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# STEROIDAL SAPONINS FROM THE BULBS OF *CAMASSIA CUSICKII*

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**Key Word Index**—*Camassia cusickii*; Liliaceae; bulbs; steroidal saponins; chlorogenin; chlorogenin glycosides.

**Abstract**—Six new steroidal saponins have been isolated from the fresh bulbs of *Camassia cusickii*. Their structures were determined by spectroscopic analysis and some chemical transformations to be (25*R*)-5α-spirostan-3β,6α-diol (chlorogenin) 6-*O*-β-D-glucopyranoside, chlorogenin 6-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, chlorogenin 6-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside, chlorogenin 6-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside, (25*R*)-6α-hydroxy-5α-spirostan-3-one 6-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside and (25*R*)-3,3-dimethoxy-5α-spirostan-6α-ol 6-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside. The saponins isolated were shown to contribute to the bitter taste of the bulbs.

## INTRODUCTION

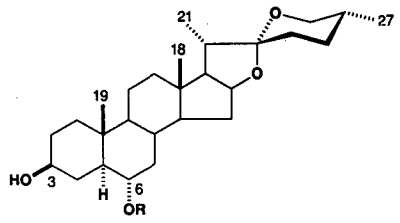
The genus *Camassia* with six species belongs to the subfamily Schilloideae in the Liliaceae and has a distribution in North America [1]. The fresh and preserved bulbs of some *Camassia* plants have been used as a nourishing food and a feed for domestic animals in North America.

*Camassia cusickii* is indigenous to the northeastern part of Oregon and bulbs have a strongly bitter taste and are not edible. Our attention to the bitter ingredients in the bulbs has resulted in finding new steroidal saponins. This paper mainly refers to the structure elucidation of the new saponins based on the spectroscopic analysis and some chemical transformations.

## RESULTS AND DISCUSSION

Commercially available fresh bulbs of *C. cusickii* were extracted with methanol to yield **1–6**. Compounds **1–6** were obtained as amorphous powders and their molecular formulas were determined by the electron impact (EI) or secondary ion (SI) mass spectra, and elemental analysis to be $C_{33}H_{54}O_9$, $C_{39}H_{64}O_{14}$, $C_{39}H_{64}O_{14}$, $C_{45}H_{74}O_{19}$, $C_{39}H_{62}O_{14}$ and $C_{41}H_{68}O_{15}$, respectively.

The $^1$H NMR spectrum of **1** showed two singlet signals at δ0.85 and 0.80, indicating the presence of two angular methyl groups, as well as two doublet signals at δ1.13 ($J$ = 6.9 Hz) and 0.72 ($J$ = 5.4 Hz) assignable to secondary methyl groups. The structure of **1** based upon a (25*R*)-spirostanol derivative was suggested by the IR spectrum [980, 915, 895 and 860 cm$^{-1}$ (intensity, 915 < 895)] [2–4], EI mass (*m/z* 139 and 115) [5], $^{13}$C NMR [δ109.2 (a quaternary carbon signal for the C-22 position)] [6] and $^1$H NMR [δ3.57 (*dd*, $J$ = 10.5, 3.0 Hz, H-26a) and 3.48 (*dd*, $J$ = 10.5, 10.5 Hz, H-26b)] spectra. In addition, the presence of a β-D-glucose moiety in **1** was readily recognized by the characteristic six signals at δ106.0, 78.7, 78.0, 75.8, 71.9 and 63.0 in the $^{13}$C NMR spectrum. When **1** was submitted to acid hydrolysis with 1 M hydrochloric acid, it was hydrolysed to yield D-glucose, identified by the TLC comparison with an authentic sample and its specific rotation, and a steroidal sapogenin (**1a**). The IR, EI mass, $^1$H and $^{13}$C NMR spectra identified **1a** as (25*R*)-5α-spirostan-3β,6α-diol, that is, chlorogenin [7, 8]. The linkage position between the β-D-glucose and the aglycone were established by the following spectral data. In

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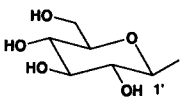


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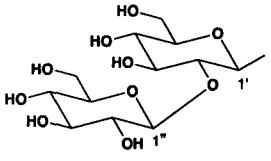


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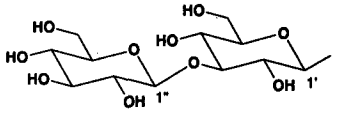


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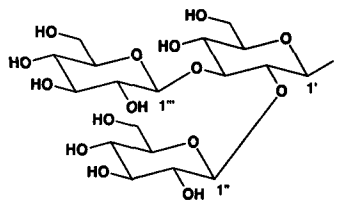

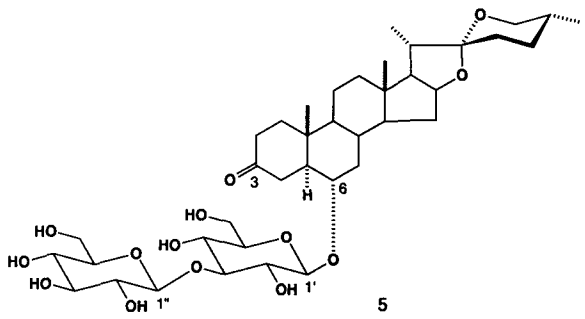

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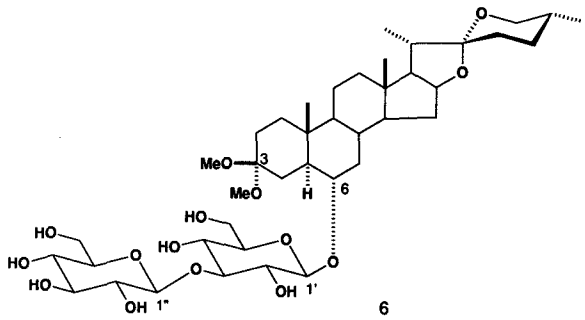

6

the $^{13}$C NMR spectrum of **1**, the signal due to C-6 was shifted to lower field by 11.1 ppm, whereas the signals due to the C-5 and C-7 moved to upper field by 1.5 and 1.4 ppm, respectively, as compared with those of **1a**. Furthermore, in the $^{1}$H NMR spectrum of the acetyl derivative (**1b**), the signal for the H-3 methine proton was shifted to lower field by *O*-acetylation to appear at $\delta$4.82 (*m*), whereas the signal for the H-6 methine proton remained unaffected ($\delta$3.51, *ddd*, $J$=11.3, 11.3, 4.7 Hz). The above facts clearly accounted for the $\beta$-D-glucose moiety linkage to the C-6 hydroxyl position. The structure of **1** was characterized as (25*R*)-5$\alpha$-spirostan-3$\beta$,6$\alpha$-diol (chlorogenin) 6-*O*-$\beta$-D-glucopyranoside.

All the spectral data proved compounds **2**–**4** also to be chlorogenin 6-*O*-glycosides. The $^{1}$H and $^{13}$C NMR spectra of **2**–**4** indicated the presence of a terminal $\beta$-D-glucosyl unit and an inner $\beta$-D-glucosyl unit in **2** and **3**, and the presence of two terminal $\beta$-D-glucosyl units and an inner $\beta$-D-glucosyl unit in **4**. The downfield shifts due to glycosidation were observed at the following signals of the inner glucose units in each compound in the $^{13}$C NMR spectra; C-2 in **2**, C-3 in **3**, and C-2 and C-3 in **4**, leading to the respective structures of the sugar moieties as $\beta$-D-glucosyl-(1→2)-$\beta$-D-glucose, $\beta$-D-glucosyl-(1→3)-$\beta$-D-glucose and $\beta$-D-glucosyl-(1→2)-*O*-[$\beta$-D-glucosyl-(1→3)]-$\beta$-D-glucose (Table 1). The $^{1}$H NMR spectra of the acetyl derivatives (**2a**–**4a**) of **2**–**4** further supported the above findings. The $\alpha$-protons of the hydroxyl groups bearing $\beta$-D-glucosyl units in the inner glucoses showed no downfield shift, whereas the other hydroxymethine and hydroxymethylene protons appeared downfield by *O*-acetylation as shown in Table 2. Thus, the structures of **2**–**4** were formulated as chlorogenin 6-*O*-$\beta$-D-glucopyranosyl-(1→2)-$\beta$-D-glucopyranoside, chlorogenin 6-*O*-$\beta$-D-glucopyranosyl-(1→3)-$\beta$-D-glucopyranoside and chlorogenin 6-*O*-$\beta$-D-glucopyranosyl-(1→2)-*O*-[$\beta$-D-glucopyranosyl-(1→3)]-$\beta$-D-glucopyranoside, respectively.

The spectral properties of **5** were essentially identical with those of **1**–**4**. The UV, IR and $^{13}$C NMR spectra showed the existence of a carbonyl group [UV: $\lambda_{max}$ 289 nm ($\varepsilon$ 102); IR: 1695 cm$^{-1}$; $^{13}$C NMR: $\delta$210.7]. Acetylation of **5** in the usual manner gave the corresponding heptaacetate (**5a**), and acid hydrolysis gave D-glucose and a steroidal sapogenin (**5b**), $C_{27}H_{42}O_4$. Compound **5b** was shown by the spectral data to be a (25*R*)-spirostan with a carbonyl group and a secondary hydroxyl group. The product (**5c**) produced by the treatment of **5b** with sodium borohydride was identified as chlorogenin. The carbonyl and secondary hydroxyl groups were determined by the $^{1}$H NMR analysis to be located at the C-3 and C-6 positions in **5b**: the chemical shifts and coupling constants of the $\alpha$- and $\beta$-protons of the hydroxyl and carbonyl groups are shown in Fig. 1. The positive Cotton effects in the CD spectrum of **5b** agreed with the presence of the C-3 carbonyl group. The structure of **5b** was deduced to be (25*R*)-6$\alpha$-hydroxy-5$\alpha$-spirostan-3-one. The signals due to the oligoside moiety were superimposable on those in the $^{1}$H and $^{13}$C NMR spectra of **3** leading to the structure of the oligoside moiety as $\beta$-D-glucosyl-(1→3)-$\beta$-D-glucose. The above facts verified that **5** is the corresponding 3-ketosteroidal saponin of **3**, and the structure is (25*R*)-6$\alpha$-hydroxy-5$\alpha$-spirostan-3-one 6-*O*-$\beta$-D-glucopyranosyl-(1→3)-$\beta$-D-glucopyranoside.

The $^{1}$H and $^{13}$C NMR spectra of **6** showed the presence of two methoxyl groups [$^{1}$H NMR: $\delta$3.24 and 3.20 (each *s*); $^{13}$C NMR: $\delta$47.5 and 47.4]. Acetylation of **6** in the usual manner gave the corresponding heptaacetate (**6a**). On treatment of **6** with 1 M hydrochloric acid at room temperature for 1 hr, *ca* 25% was converted to **5**. The above data were indicative of **6** being a ketal form of **5**, and the structure was elucidated as (25*R*)-3,3-dimethoxy-5$\alpha$-spirostan-6$\alpha$-o1 6-*O*-$\beta$-D-glucopyranosyl-(1→3)-$\beta$-D-glucopyranoside. Steroidal compounds containing the 3,3-dimethoxyl group have been previously isolated from the green berries of *Solanum pseudoquina* [9]. Compound **6** is considered to be a natural product, not an artifact produced from **5** during extraction and purification because generation of a dimethoxy functionality from a carbonyl group requires acid catalysis under anhydrous conditions.

Several steroidal saponins have been identified as the bitter ingredients of plants [10–14]. The steroidal saponins **2**–**4**, which were obtained in good yields, have a bitter taste and are considered to contribute the bitter taste of the bulbs of *C. cusickii*.

## EXPERIMENTAL

*General.* $^{1}$H NMR (400 MHz) and $^{13}$C NMR (100.6 MHz): TMS as int. standard. Assignments of the NMR spectra were achieved on the basis of the double resonance experiments, $^{1}$H–$^{1}$H COSY, $^{13}$C DEPT spectra, and by the correlation with previously reported compounds. CC: silica gel (Fuji Davison), Sephadex LH-20 (Pharmacia Fine Chemicals) and Diaion HP-20 (Mitsubishi-kasei). HPLC: Tosoh HPLC system (pump, Tosoh CCPM; detector, Tosoh RI-8010; controller, CCP controller PX-8010) equipped with a CIG pre-packed column (Kusano Kagakukikai, 22 i.d. × 100 mm, ODS 20 $\mu$m) for prep. HPLC and with a Kaseisorb LC ODS-120-5 column (Tokyo-kasei, 4.6 i.d. × 250 mm, ODS 5 $\mu$m) for analytical HPLC.

*Extraction and isolation.* Fresh bulbs of *Camassia cusickii* (4.4 kg) purchased from Heiwaen, Japan, were cut into pieces and extracted with MeOH under reflux. The extract was concd to

Table 1. $^{13}C$ NMR spectral data for compounds **1**, **1a**, **2**, **3**, **4**, **5**, **5b** and **6** (pyridine-$d_5$)

| C | 1 | 1a | 2 | 3 | 4 | 5 | 5b ($CDCl_3$) | 6 |
|---|---|---|---|---|---|---|---|---|
| 1 | 37.8 | 38.1 | 37.9 | 37.8 | 37.9 | 39.9[a] | 39.5[a] | 35.7 |
| 2 | 32.3[a] | 32.4[a] | 32.2[a] | 32.2[a] | 32.2[a] | 38.7[a] | 38.5[a] | 30.1[a] |
| 3 | 70.7 | 71.0 | 70.9 | 70.7 | 71.1 | 210.7 | 211.3 | 100.8 |
| 4 | 33.2[a] | 33.8[a] | 32.4[a] | 33.3[a] | 32.7[a] | 38.1[a] | 37.8[a] | 28.9[a] |
| 5 | 51.3 | 52.8 | 51.0 | 51.3 | 51.0 | 52.3 | 53.1[b] | 48.9 |
| 6 | 79.7 | 68.6 | 80.7 | 79.8 | 80.3 | 80.4 | 69.8 | 79.8 |
| 7 | 41.5 | 42.9 | 41.0 | 41.4 | 40.6 | 40.7 | 41.7 | 41.2 |
| 8 | 34.2 | 34.4 | 34.1 | 34.2 | 34.0 | 34.0 | 33.9 | 34.1 |
| 9 | 54.0 | 54.3 | 54.0 | 53.9 | 54.0 | 53.3 | 53.3[b] | 53.6 |
| 10 | 36.7 | 36.6 | 36.7 | 36.7 | 36.6 | 36.8 | 36.6 | 36.8 |
| 11 | 21.3 | 21.4 | 21.3 | 21.3 | 21.3 | 21.3 | 21.1 | 21.2 |
| 12 | 40.1 | 40.2 | 40.1 | 40.1 | 40.1 | 39.9 | 39.7 | 40.1 |
| 13 | 40.8 | 40.9 | 40.8 | 40.8 | 40.8 | 40.9 | 40.6 | 40.8 |
| 14 | 56.5 | 56.5 | 56.5 | 56.4 | 56.4 | 56.2 | 55.8 | 56.4 |
| 15 | 32.1 | 32.2 | 32.1 | 32.1 | 32.1 | 32.0 | 31.8 | 32.0 |
| 16 | 81.1 | 81.1 | 81.1 | 81.0 | 81.0 | 81.0 | 80.6 | 81.0 |
| 17 | 63.0 | 63.1 | 63.0 | 63.0 | 63.0 | 63.0 | 62.2 | 63.0 |
| 18 | 16.7 | 16.7 | 16.6 | 16.7 | 16.6 | 16.6 | 16.4 | 16.7 |
| 19 | 13.6 | 13.8 | 13.7 | 13.6 | 13.7 | 12.6 | 12.8 | 12.9 |
| 20 | 42.0 | 42.0 | 42.0 | 42.0 | 42.0 | 42.0 | 41.7 | 42.0 |
| 21 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 14.5 | 15.0 |
| 22 | 109.2 | 109.2 | 109.2 | 109.2 | 109.1 | 109.2 | 109.3 | 109.1 |
| 23 | 31.9 | 31.8 | 31.8 | 31.9 | 31.8 | 31.8 | 31.4 | 31.8 |
| 24 | 29.3 | 29.3 | 29.3 | 29.3 | 29.3 | 29.3 | 28.8 | 29.3 |
| 25 | 30.6 | 30.6 | 30.6 | 30.6 | 30.6 | 30.6 | 30.3 | 30.6 |
| 26 | 66.9 | 66.9 | 66.9 | 66.9 | 66.8 | 66.9 | 66.9 | 66.9 |
| 27 | 17.4 | 17.3 | 17.3 | 17.4 | 17.3 | 17.4 | 17.1 | 17.4 |
| 1′ | 106.0 | | 103.7 | 105.5 | 104.7[b] | 105.5 | | 105.9[b] |
| 2′ | 75.8 | | 84.6 | 74.5 | 79.7 | 74.4 | | 74.3 |
| 3′ | 78.7[b] | | 77.9 | 89.1 | 89.3 | 88.9 | | 88.9 |
| 4′ | 71.9 | | 71.4[b] | 69.8 | 70.1 | 69.8 | | 69.9 |
| 5′ | 78.0[b] | | 79.0 | 77.7 | 78.6[c] | 77.8 | | 77.7 |
| 6′ | 63.0 | | 62.2 | 62.6[b] | 62.6[d] | 62.5 | | 62.6 |
| 1″ | | | 106.3 | 106.1 | 103.9[b] | 106.0 | | 105.8[b] |
| 2″ | | | 76.6 | 75.7 | 76.1[e] | 75.7 | | 75.6 |
| 3″ | | | 78.5[c] | 78.7[c] | 78.6[c] | 78.7[b] | | 78.6[c] |
| 4″ | | | 71.3[b] | 71.7 | 71.6 | 71.7 | | 71.7 |
| 5″ | | | 78.4[c] | 78.3[c] | 77.6[c] | 78.3[b] | | 78.3[c] |
| 6″ | | | 62.8 | 62.5[b] | 62.5[d] | 62.5 | | 62.6 |
| 1‴ | | | | | 103.4[b] | | | |
| 2‴ | | | | | 75.5[e] | | | |
| 3‴ | | | | | 78.6[c] | | | |
| 4‴ | | | | | 71.6 | | | |
| 5‴ | | | | | 77.6[c] | | | |
| 6‴ | | | | | 62.4[d] | | | 47.5 |
| OMe | | | | | | | | 47.4 |

[a–c]May be interchangeable within in each column.

almost dryness under red. pres., and the crude residue, after dilution with $H_2O$, was extracted with *n*-BuOH. The *n*-BuOH sol-phase was fractionated on a silica gel column with a gradient mixt. of $CH_2Cl_2$–MeOH. Frs with the same TLC profile were combined. Six frs were recovered. Frs 3–6 mainly contained steroidal saponins. Fr. 3 was chromatographed on a silica gel column, eluated with $CHCl_3$–MeOH–$H_2O$ (140:20:1) and EtOAc–MeOH (9:1) solvent systems, and on Sephadex LH-20 with MeOH to yield compounds **1** and **6** with a few impurities. Final purification of **1** and **6** was carried out by prep. HPLC with MeOH–$H_2O$ (19:1). The removal of saccharides from the fr. 4 was performed by CC on Diaion HP-20 with an increasing amount of MeOH in $H_2O$. Chromatography of the MeOH eluate fr. on silica gel with $CHCl_3$–MeOH–$H_2O$ (100:20:1) and EtOAc–MeOH (7:1) and on Sephadex LH-20 with MeOH to collect a mixt. of **2** and **3**. Separation of **2** and **3** was carried out by means of prep. HPLC with MeOH–$H_2O$ (9:1). Fr. 5 was subjected to Diaion HP-20 CC using gradients of MeOH in $H_2O$ as the solvent. Compound **4** was isolated from the MeOH eluate fr. after purification on Sephadex LH-20 CC with MeOH and on

Table 2. $^1$H NMR spectral data for compounds **1b**, **2a**, **3a**, **4a**, **5a** and **6a** (chloroform-*d*)

| H | **1b** (pyridine-$d_5$) | **2a** | **3a** | **4a** (benzene-$d_6$) | **5a** | **6a** |
|---|---|---|---|---|---|---|
| 3 | 4.82 *m* | 4.72 *m* | 4.63 *m* | 5.06 *m* | | |
| 6 | 3.51 *ddd* (11.3, 11.3, 4.7) | 4.27 *ddd* (10.0, 10.0, 4.0) | 3.23 *ddd* (10.4, 10.4, 4.4) | 3.24 *ddd* (10.2, 10.2, 3.5) | 3.29 *ddd* (10.8, 10.8, 4.6) | 3.22 *ddd* (10.9, 10.9, 4.5) |
| 16 | 4.53 *q*-like (7.3) | 4.38 overlapping | 4.38 *q*-like (7.1) | 4.55 *q*-like (7.0) | 4.38 *q*-like (7.2) | 4.38 overlapping |
| 18 | 0.79 *s* | 0.75 *s* | 0.74 *s* | 0.72 *s* | 0.77 *s* | 0.74 *s* |
| 19 | 0.84 *s* | 0.86 *s* | 0.84 *s* | 0.81 *s* | 1.02 *s* | 0.80 *s* |
| 21 | 1.14 *d* (6.9) | 0.96 *d* (6.9) | 0.95 *d* (6.9) | 1.20 *d* (6.9) | 0.96 *d* (6.9) | 0.95 *d* (6.9) |
| 26 | 3.57 *dd* (11.3, 3.2) | 3.46 *dd* (10.9, 3.7) | 3.46 *dd* (10.9, 3.6) | 3.55 | 3.46 *dd* (10.9, 3.5) | 3.46 *dd* (11.0, 4.1) |
| | 3.47 *dd* (11.3, 11.3) | 3.36 *dd* (10.9, 10.9) | 3.36 *dd* (10.9, 10.9) | | 3.36 *dd* (10.9, 10.9) | 3.35 *dd* (11.0, 11.0) |
| 27 | 0.70 *d* (5.4) | 0.78 *d* (6.3) | 0.78 *d* (6.3) | 0.64 *d* (6.0) | 0.78 *d* (7.3) | 0.78 *d* (6.3) |
| 1′ | 4.97 *d* (8.0) | 4.37 *d* (7.8) | 4.36 *d* (8.1) | 4.22 *d* (7.3) | 4.34 *d* (8.2) | 4.37 *d* (8.1) |
| 2′ | 5.46 *dd* (9.6, 8.0) | 3.72 *dd* (9.5, 7.8) | 4.97 *dd* (9.5, 8.1) | 4.04 *dd* (9.4, 7.3) | 4.98 *dd* (9.5, 8.2) | 5.01 *dd* (9.5, 8.1) |
| 3′ | 5.71 *dd* (9.6, 9.6) | 5.13 *dd* (9.5, 9.5) | 3.87 *dd* (9.5, 9.5) | 4.09 *dd* (9.4, 9.4) | 3.85 *dd* (9.5, 9.5) | 3.84 *dd* (9.5, 9.5) |
| 4′ | 5.47 *dd* (9.6, 9.6) | 4.91 *dd* (9.5, 9.5) | 4.90 *dd* (9.5, 9.5) | 5.21 *dd* (9.4, 9.4) | 4.90 *dd* (9.5, 9.5) | 4.91 *dd* (9.5, 9.5) |
| 5′ | 4.13 *ddd* (9.6, 4.8, 2.1) | 3.63 *ddd* (9.5, 4.5, 2.3) | 3.64 *ddd* (9.5, 4.6, 2.5) | 3.36 *ddd* (9.4, 4.9, 2.5) | 3.64 *ddd* (9.5, 4.5, 2.6) | 3.63 *ddd* (9.5, 4.7, 2.5) |
| 6′ | 4.58 *dd* (12.2, 4.8) | 4.30 *dd* (12.3, 4.5) | 4.20 *dd* (12.2, 4.6) | 4.28 *dd* (12.1, 4.9) | 4.20 *dd* (12.2, 4.5) | 4.20 *dd* (12.2, 4.7) |
| | 4.44 *dd* (12.2, 2.1) | 4.03 *dd* (12.3, 2.3) | 4.11 *dd* (12.2, 2.5) | 4.17 *dd* (12.1, 2.5) | 4.12 *dd* (12.2, 2.6) | 4.10 *dd* (12.2, 2.5) |
| 1″ | | 4.60 *d* (8.1) | 4.57 *d* (8.1) | 5.21 *d* (7.7) | 4.57 *d* (8.1) | 4.56 *d* (8.1) |
| 2″ | | 4.87 *dd* (9.6, 8.1) | 4.88 *dd* (9.4, 8.1) | 5.44 *dd* (9.1, 7.7) | 4.89 *dd* (9.3, 8.1) | 4.89 *dd* (9.3, 8.1) |
| 3″ | | 5.14 *dd* (9.6, 9.6) | 5.12 *dd* (9.4, 9.4) | 5.60 *dd* (9.1, 9.1) | 5.12 *dd* (9.3, 9.3) | 5.12 *dd* (9.3, 9.3) |
| 4″ | | 5.10 *dd* (9.6, 9.6) | 5.05 *dd* (9.4, 9.4) | 5.65 *dd* (9.1, 9.1) | 5.06 *dd* (9.3, 9.3) | 5.05 *dd* (9.3, 9.3) |
| 5″ | | 3.64 *m* | 3.66 *ddd* (9.4, 4.0, 2.3) | 3.90 *ddd* (9.1, 5.7, 2.4) | 3.67 *ddd* (9.3, 4.1, 2.3) | 3.67 *ddd* (9.3, 4.6, 2.2) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6″ | | 4.38 overlapping<br>4.07 *dd* (12.2, 2.5) | 4.36 *dd* (12.4, 4.0)<br>4.04 *dd* (12.4, 2.3) | 4.80 *dd* (12.2, 5.7)<br>4.25 *dd* (12.2, 2.4) | 4.36 *dd* (12.3, 4.1)<br>4.04 *dd* (12.3, 2.3) | 4.37 *dd* (12.4, 4.6)<br>4.05 *dd* (12.4, 2.2) |
| 1‴ | | | | 5.13 *d* (7.2) | | |
| 2‴ | | | | 5.35 *dd* (8.9, 7.2) | | |
| 3‴ | | | | 5.48 *dd* (8.9, 8.9) | | |
| 4‴ | | | | 5.31 *dd* (8.9, 8.9) | | |
| 5‴ | | | | 3.65 *ddd* (8.9, 4.6, 2.0) | | |
| 6‴ | | | | 4.47 *dd* (12.5, 4.6)<br>4.00 *dd* (12.5, 2.0) | | |
| Ac | 2.24 | 2.13 | 2.09 | 2.27 | 2.21 | 2.14 |
| | 2.04 × 2 | 2.12 | 2.08 | 2.03 | 2.09 | 2.09 |
| | 2.01 | 2.09 | 2.07 | 2.00 | 2.07 | 2.08 |
| | 2.00 | 2.07 | 2.02 × 2 | 1.97 | 2.02 | 2.02 |
| | | 2.00 | 2.01 | 1.84 | 2.01 | 2.01 |
| | | 1.99 × 2 | 2.00 | 1.83 | 2.00 | 2.00 |
| | | 1.97 | 1.98 | 1.77 | 1.98 | 1.98 |
| | | | | 1.74 | | |
| | | | | 1.73 | | |
| | | | | 1.67 | | |
| | | | | 1.66 | | |
| OMe | | | | | | 3.91 |
| | | | | | | 3.06 |

*J* values in parentheses are expressed in Hz. All the assignments were confirmed by the double resonance experiments. In **4a**, signals for the H-1″–H-6″ were interchangeable with those for the H-1‴–H-6‴.

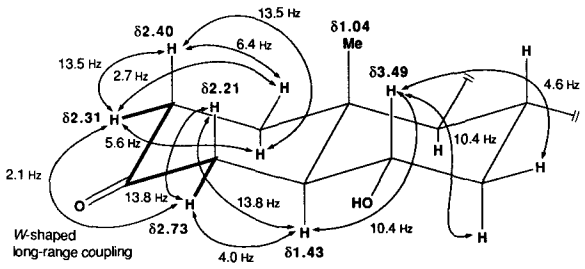


Fig. 1.

silica gel CC with EtOAc–MeOH (5:1) and $CHCl_3$–MeOH–$H_2O$ (80:20:1). Further analysis of the frs 5 and 6 is now under way.

*Compound* **1**. Amorphous powder (200 mg), $[\alpha]_D^{25}$ −26.1° (MeOH; *c* 0.36) (Found: C, 64.23; H, 9.09. Calc. for $C_{33}H_{54}O_9 \cdot H_2O$: C, 64.68; H, 9.21%). EIMS *m/z* (rel. int.): 594 $[M]^+$ (0.6), 535 (0.8), 522 (0.8), 433 (1.4), 432 (1.4), 415 (13), 301 (7), 271 (7), 139 (100), 115 (20), 107 (11); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420 (OH), 2950, 2930, 2875 (CH), 1450, 1375, 1340, 1300, 1240, 1170, 1160, 1075, 1045, 980, 955, 915, 895, 860 (intensity 915 < 895, 25*R*-spiroketal); $^1H$ NMR (pyridine-$d_5$): δ4.94 (H-1′, overlapping with $H_2O$ signal), 4.53–4.35 (3H, $H_2$-6′ and H-16), 4.25 (2H, H-3′ and H-4′), 4.54 (1H, *dd*, *J* = 8.0, 8.0 Hz, H-2′), 3.93 (1H, *m*, H-5′), 3.78 (1H, *m*, H-3), 3.73 (1H, *ddd*, *J* = 10.7, 10.7, 4.6 Hz, H-6), 3.57 (1H, *dd*, *J* = 10.5, 3.0 Hz, H-26a), 3.48 (1H, *dd*, *J* = 10.5, 10.5 Hz, H-26b), 3.26 (1H, *br d*, *J* = 12.3 Hz, H-4eq), 2.62 (1H, *ddd*, *J* = 12.5, 4.1, 4.1 Hz, H-7eq), 1.94 (1H, *m*, H-20), 1.13 (3H, *d*, *J* = 6.9 Hz, H-21), 0.85 (3H, *s*, H-18 or H-19), 0.80 (3H, *s*, H-18 or H-19), 0.72 (3H, *d*, *J* = 5.4 Hz, H-27), 0.61 (1H, *ddd*, *J* = 11.8, 11.8, 3.8 Hz, H-9).

*Acid hydrolysis of compound* **1**. Hydrolysis of **1** (60.1 mg) with 1 M HCl ($H_2O$–dioxane, 1:1) was carried out at 100° for 1 hr under an $N_2$ atmosphere. The reaction mixt., after cooling, was neutralized with 1 M NaOH and chromatographed on silica gel with $CHCl_3$–$Me_2CO$ (4:1) and $CHCl_3$–MeOH–$H_2O$ (40:20:1) to yield D-glucose (11.2 mg) and an aglycone (31.5 mg) (**1a**), identified as chlorogenin. D-Glucose: $[\alpha]_D^{25}$ +41.4° ($H_2O$; *c* 0.58); TLC, $R_f$ 0.39 (*n*-BuOH–$Me_2CO$–$H_2O$, 4:5:1). Chlorogenin (**1a**): $[\alpha]_D^{25}$ −50.0° ($CHCl_3$–MeOH, 1:2; *c* 0.30). EIMS *m/z* (rel. int.): 432 $[M]^+$ (3), 360 (9), 318 (7), 289 (11), 184 (11), 139 (100), 115 (17); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3520, 3250 (OH), 2955, 2930, 2875, 2850 (CH), 1450, 1375, 1340, 1240, 1175, 1075, 1055, 1015, 1005, 980, 955, 915, 895, 865 (intensity 915 < 895, 25*R*-spiroketal); $^1H$ NMR (pyridine-$d_5$): δ4.55 (1H, *q*-like, *J* = 6.9 Hz, H-16), 3.93 (1H, *m*, H-3), 3.67 (1H, *m*, H-6), 3.59 (1H, *dd*, *J* = 10.5, 3.6 Hz, H-26a), 3.50 (1H, *dd*, *J* = 10.5, 10.5 Hz, H-26b), 3.01 (1H, *br d*, *J* = 12.4 Hz, H-4eq), 2.25 (1H, *ddd*, *J* = 12.1, 4.1, 4.1 Hz, H-7eq), 1.98 (1H, *m*, H-20), 1.15 (3H, *d*, *J* = 7.0 Hz, H-21), 0.89 (3H, *s*, H-18 or H-19), 0.87 (3H, *s*, H-18 or H-19), 0.70 (3H, *d*, *J* = 5.7 Hz, H-27).

*Compound* **2**. Amorphous powder (4.16 g), $[\alpha]_D^{25}$ −20.0° (MeOH; *c* 0.26) (Found: C, 59.95; H, 8.49. Calc. for $C_{39}H_{64}O_{14} \cdot H_2O$: C, 60.45; H, 8.58%). SIMS *m/z* 779 $[M+Na]^+$, 757 $[M+H]^+$, 595; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400 (OH), 2920, 2865 (CH), 1445, 1370, 1235, 1165, 1150, 1065, 1045, 975, 945, 910, 890, 855 (intensity 910 < 890, 25*R*-spiroketal); $^1H$ NMR (pyridine-$d_5$): δ5.41 (1H, *d*, *J* = 7.4 Hz, H-1″), 4.91 (H-1′, overlapping with $H_2O$ signal), 3.87 (1H, *m*, H-3), 3.63 (1H, *ddd*, *J* = 10.6, 10.6, 4.1 Hz, H-6), 3.58 (1H, *dd*, *J* = 10.4, 1.8 Hz, H-26a), 3.47 (1H, *dd*, *J* = 10.4, 10.4 Hz, H-26b), 3.42 (1H, *br d*, *J* = 13.3 Hz, H-4eq), 2.63 (1H, *ddd*, *J* = 12.7, 3.6, 3.6 Hz, H-7eq), 2.03 (1H, *br d*, *J* = 11.6 Hz, H-2eq), 1.95 (1H, *m*, H-20), 1.13 (3H, *d*, *J* = 6.9 Hz, H-21), 0.82 (3H, *s*, H-18 or H-19), 0.80 (3H, *s*, H-18 or H-19), 0.73 (3H, *d*, *J* = 5.0 Hz, H-27), 0.62 (1H, *ddd*, *J* = 10.6, 10.6, 3.6 Hz, H-9).

*Compound* **3**. Amorphous powder (5.45 g), $[\alpha]_D^{25}$ −20.0° (MeOH; *c* 0.21) (Found: C, 60.27; H, 8.54. Calc. for $C_{39}H_{64}O_{14} \cdot H_2O$: C, 60.45; H, 8.58%). SIMS *m/z* 779 $[M+Na]^+$, 757 $[M+H]^+$, 595; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3410 (OH), 2935, 2875 (CH), 1450, 1375, 1340, 1300, 1260, 1170, 1155, 1075, 1050, 980, 965, 920, 895, 865 (intensity 920 < 895, 25*R*-spiroketal); $^1H$ NMR (pyridine-$d_5$): δ5.30 (1H, *d*, *J* = 7.8 Hz, H-1″), 4.89 (H-1′, overlapping with $H_2O$ signal), 3.80 (1H, *m*, H-3), 3.72 (1H, *ddd*, *J* = 10.8, 10.8, 4.5 Hz, H-6), 3.57 (1H, *dd*, *J* = 10.5, 2.6 Hz, H-26a), 3.47 (1H, *dd*, *J* = 10.5, 10.5 Hz, H-26b), 3.22 (1H, *br d*, *J* = 12.2 Hz, H-4eq), 2.58 (1H, *ddd*, *J* = 12.7, 3.6, 3.6 Hz, H-7eq), 2.04 (1H, *br d*, *J* = 11.7 Hz, H-2eq), 1.94 (1H, *m*, H-20), 1.13 (3H, *d*, *J* = 6.9 Hz, H-21), 0.84 (3H, *s*, H-18 or H-19), 0.80 (3H, *s*, H-18 or H-19), 0.72 (3H, *d*, *J* = 5.3 Hz, H-27), 0.60 (1H, *ddd*, *J* = 11.8, 11.8, 3.4 Hz, H-9).

*Compound* **4**. Amorphous powder (3.93 g), $[\alpha]_D^{25}$ −12.0° ($CHCl_3$ – MeOH, 1:3; *c* 0.20) (Found: C, 57.48; H, 8.06. Calc. for $C_{45}H_{74}O_{19} \cdot H_2O$: C, 57.68; H, 8.17%). SIMS *m/z* 941 $[M+Na]^+$, 918 $[M]^+$, 757, 645; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3370 (OH), 2930 (CH), 1445, 1370, 1235, 1150, 1070, 1050, 1030, 975, 950, 915, 895, 860 (intensity 915 < 895, 25*R*-spiroketal); $^1H$ NMR (pyridine-$d_5$): δ5.77 (1H, *d*, *J* = 7.7 Hz, H-1″ or H-1‴), 5.40 (1H, *d*, *J* = 7.8 Hz, H-1″ or H-1‴), 4.82 (1H, *d*, *J* = 7.5 Hz, H-1′), 3.90 (1H, *m*, H-3), 3.65 (1H, *ddd*, *J* = 10.6, 10.6, 4.3 Hz, H-6), 3.57 (1H, *br d*, *J* = 10.2 Hz, H-26a), 3.47 (1H, *dd*, *J* = 10.2, 10.2 Hz, H-26b), 3.39 (1H, *br d*, *J* = 12.3 Hz, H-4eq), 2.48 (1H, *br d*, *J* = 12.3 Hz, H-7eq), 2.01 (1H, *br d*, *J* = 11.0 Hz, H-2eq), 1.94 (1H, *m*, H-20), 1.13 (3H, *d*, *J* = 6.8 Hz, H-21), 0.83 (3H, *s*, H-18 or H-19), 0.81 (3H, *s*, H-18 or H-19), 0.72 (3H, *d*, *J* = 4.8 Hz, H-27), 0.56 (1H, *ddd*, *J* = 11.2, 11.2, 2.8 Hz, H-9).

*Compound* **5**. Amorphous powder (24.2 mg), $[\alpha]_D^{25}$ −22.3° (MeOH; *c* 0.26) (Found: C, 60.26; H, 8.28. Calc. for $C_{39}H_{62}O_{14} \cdot H_2O$: C, 60.60; H, 8.35%). SIMS *m/z* 777 $[M+Na]^+$, 755 $[M+H]^+$, 595, 431; UV $\lambda_{max}^{MeOH}$ nm (ε): 289 (102); CD (MeOH; *c* 9.55 × $10^{-4}$) nm (θ): 289 (+1099); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420 (OH), 2950, 2875 (CH), 1695 (C=O), 1450, 1375, 1255, 1235, 1170, 1155, 1075, 1040, 980, 960, 915, 895, 860 (intensity 915 < 895, 25*R*-spiroketal); $^1H$ NMR (pyridine-$d_5$): δ5.32 (1H, *d*, *J* = 7.8 Hz, H-1″), 4.83 (1H, *d*, *J* = 7.8 Hz, H-1′), 3.71 (1H, *ddd*, *J* = 10.6, 10.6, 4.4 Hz, H-6), 3.57 (2H, overlapping, H-4eq and H-26a), 3.48 (1H, *dd*, *J* = 10.5, 10.5 Hz, H-26b), 2.57 (1H, *ddd*, *J* = 12.7, 3.9, 3.9 Hz, H-7eq), 2.46 (1H, *dd*, *J* = 14.5, 14.5, H-4ax), 2.42 (1H, *ddd*, *J* = 14.8, 14.8, 6.4 Hz, H-2ax), 2.31 (1H, *br d*, *J* = 14.8 Hz, H-2eq), 1.94 (1H, *m*, H-20), 1.13 (3H, *d*, *J* = 6.8 Hz, H-21), 0.97 (3H, *s*, H-19), 0.82 (3H, *s*, H-18), 0.73 (3H, *d*, *J* = 5.3 Hz, H-27), 0.61 (1H, *ddd*, *J* = 11.0, 11.0, 3.4 Hz, H-9).

*Acid hydrolysis of compound* **5**. Compound **5** (10.0 mg) was subjected to acid hydrolysis as for **1** to yield D-glucose and a sapogenin (**5b**) (3.5 mg). Compound **5b**: amorphous powder, $[\alpha]_D^{25}$ −37.4° ($CHCl_3$; *c* 0.23). EIMS *m/z* (rel. int.): 430 $[M]^+$ (5), 358 (9), 316 (10), 287 (15), 139 (100), 115 (25); UV $\lambda_{max}^{EtOH}$ nm (ε): 286 (201); CD (EtOH; *c* 1.12 × $10^{-3}$) nm (θ): 290 (+2813); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3455 (OH), 2965, 2930, 2910, 2865 (CH), 1690 (C=O), 1450, 1390, 1375, 1350, 1340, 1275, 1240, 1180, 1175, 1145, 1090, 1070, 1050, 1015, 1005, 985, 980, 965, 960, 920, 900, 865 (intensity 920 < 900, 25*R*-spiroketal); $^1H$ NMR (chloroform-$d_1$): δ4.41 (1H, *q*-like, *J* = 7.2 Hz, H-16), 3.49 (1H, *ddd*, *J* = 10.4, 10.4, 4.6 Hz, H-6), 3.48 (1H, *br d*, *J* = 10.9 Hz, H-26a), 3.37 (1H, *dd*, *J* = 10.9, 10.9 Hz, H-26b), 2.73 (1H, *ddd*, *J* = 13.8, 4.0, 2.1 Hz, H-4eq), 2.40 (1H, *ddd*, *J* = 13.5, 13.5, 6.4 Hz, H-2ax), 2.31 (1H, *dddd*, *J* = 13.5, 5.6, 2.7, 2.1 Hz, H-2eq), 2.21 (1H, *dd*, *J* = 13.8, 13.8 Hz, H-4ax), 1.04 (3H, *s*, H-19), 0.97 (3H, *d*, *J* = 6.9 Hz, H-21), 0.79 (3H, *s*, H-18), 0.78 (3H, *d*, *J* = 6.3 Hz, H-27).

$NaBH_4$ *reduction of compound* **5b**. A mixt. of **5b** (2.8 mg)

dissolved in EtOH with $NaBH_4$ (10.0 mg) was allowed to stand at room temp. for 1 hr. Purification of the reaction mixt. was carried out by silica gel CC with $CHCl_3$–$Me_2CO$ (3:1) to give a product (1.8 mg), identified as chlorogenin (**1b**).

*Compound* **6**. Amorphous powder (49.2 mg), $[\alpha]_D^{25}$ −29.0° (MeOH; *c* 0.20) (Found: C, 59.31; H, 8.66. Calc. for $C_{41}H_{68}O_{15}\cdot 3/2H_2O$: C, 59.47; H, 8.64%). SIMS *m/z* 823 $[M+Na]^+$, 799 $[M-H]^+$; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3430 (OH), 2945 (CH), 1445, 1370, 1335, 1235, 1170, 1150, 1070, 1035, 975, 945, 915, 890, 870, 860 (intensity 915 < 890, 25*R*-spiroketal); $^1H$ NMR (pyridine-$d_5$): δ 5.30 (1H, *d*, *J* = 7.8 Hz, H-1″), 4.86 (1H, *d*, *J* = 7.8 Hz, H-1′), 3.65 (1H, *ddd*, *J* = 10.9, 10.9, 4.4 Hz, H-6), 3.57 (1H, *dd*, *J* = 10.6, 2.8 Hz, H-26a), 3.47 (1H, *dd*, *J* = 10.6, 10.6 Hz, H-26b), 3.24 and 3.20 (each 3H, *s*, OMe × 2), 2.53 (1H, *ddd*, *J* = 12.5, 4.0, 4.0 Hz, H-7eq), 1.12 (3H, *d*, *J* = 6.9 Hz, H-21), 0.80 (3H, *s*, H-18 or H-19), 0.79 (3H, *s*, H-18 or H-19), 0.72 (3H, *d*, *J* = 5.5 Hz, H-27), 0.64 (1H, *ddd*, *J* = 11.4, 11.4, 3.7 Hz, H-9).

*Acid treatment of compound* **6**. Compound **6** (2.0 mg) was treated with 1 M HCl (MeOH–dioxane, 1:1) at room temp. for 1 hr. After neutralization with 1 M NaOH, the reaction mixt. was submitted to HPLC analysis (MeOH–$H_2O$, 19:1; 0.55 ml $min^{-1}$). *Ca* 25% of **6** was converted to **5**.

*Acetylation of compounds* **1–6**. Compounds **1** (17.3 mg), **2** (14.0 mg), **3** (15.6 mg), **4** (30.2 mg), **5** (4.0 mg), and **6** (10.4 mg) were acetylated with $Ac_2O$ in pyridine. Usual work-up and chromatography on silica gel with *n*-hexane–$Me_2CO$ or $CHCl_3$–EtOAc to give the corresponding peracetates, **1b** (17.6 mg), **2a** (19.6 mg), **3a** (19.4 mg), **4a** (32.5 mg), **5a** (4.6 mg) and **6a** (13.7 mg) as amorphous powders. Compound **1b**: $[\alpha]_D^{25}$ −47.5° ($CHCl_3$; *c* 0.24). CIMS *m/z* (rel. int.): 805 $[M+H]^+$ (5), 457 (14), 397 (38), 331 (100), 271 (21), 211 (7), 169 (62), 139 (77), 115 (23), 109 (23); IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2950 (CH), 1755 (C=O), 1445, 1375, 1365, 1245, 1230, 1175, 1080, 1045, 980, 955, 915, 895, 860 (intensity 915 < 895, 25*R*-spiroketal). Compound **2a**: $[\alpha]_D^{25}$ −31.9° ($CHCl_3$; *c* 0.47). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2960, 2880 (CH), 1755 (C=O), 1450, 1370, 1240, 1175, 1035, 980, 955, 915, 895, 860 (intensity 915 < 895, 25*R*-spiroketal). Compound **3a**: $[\alpha]_D^{25}$ −41.1° ($CHCl_3$; *c* 0.71). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2955, 2880 (CH), 1755 (C=O), 1450, 1430, 1375, 1230, 1175, 1160, 1040, 980, 955, 915, 895, 865 (intensity 915 < 895, 25*R*-spiroketal). Compound **4a**: $[\alpha]_D^{25}$ −27.3° ($CHCl_3$; *c* 0.33). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2950, 2875 (CH), 1750 (C=O), 1445, 1430, 1370, 1220, 1175, 1040, 980, 955, 915, 895, 860 (intensity 915 < 895, 25*R*-spiroketal). Compound **5a**: $[\alpha]_D^{25}$ −46.0° ($CHCl_3$; *c* 0.67). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2950 (CH), 1750, 1705 (C=O), 1445, 1370, 1220, 1175, 1040, 980, 955, 915, 895, 860 (intensity 915 < 895, 25*R*-spiroketal). Compound **6a**: $[\alpha]_D^{25}$ −45.4° ($CHCl_3$; *c* 0.26). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2950 (CH), 1745 (C=O), 1445, 1365, 1220, 1170, 1150, 1090, 1035, 985, 945, 910, 890, 865 (intensity 910 < 890, 25*R*-spiroketal).

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