New Steroidal Constituents of the Bulbs of *Camassia cusickii* S. WATS.

Yoshihiro MIMAKI, Yutaka SASHIDA* and Kazuhiro KAWASHIMA

Tokyo College of Pharmacy, 1432–1, Horinouchi, Hachioji, Tokyo 192–03, Japan. *Received July 26, 1991*

Seven new steroidal compounds were isolated from the fresh bulbs of *Camassia cusickii* S. WATS. Their structures were determined by spectroscopic analysis and hydrolysis to be (25*R*)-5α-spirostan-3β,6α-diol (chlorogenin) 6-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranoside (1), chlorogenin 6-*O*-β-D-xylopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (2), chlorogenin 6-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranoside (3), chlorogenin 6-*O*-β-D-fucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (4), 22-*O*-methyl-26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-3β,6α,22ξ-triol 6-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (5), 22-*O*-methyl-26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-3β,6α,22ξ-triol 6-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (6) and 3β,7β,16β-trihydroxycholest-5-en-23-one 3,16-bis-*O*-β-D-glucopyranoside (camassioside) (7).

Keywords *Camassia cusickii*; Liliaceae; steroidal glycoside; spirostanol glycoside; furostanol glycoside; chlorogenin glycoside; camassioside; bulb

The family Liliaceae is well known for the presence of steroidal saponins.[1] We previously examined the bulbs of *Camassia cusickii* and isolated six new steroidal saponins as the bitter principles.[2] Further investigation of the methanolic extract of the bulbs of *C. cusickii* led to the isolation of seven new steroidal glycosides. This paper gives an account of their structural elucidation.

The methanolic extract of the bulbs was further fractionated through the combined use of repeated silica gel and octadecylanized (ODS) silica gel column chromatographies, and then preparative high-performance liquid chromatography (HPLC) resulted in the isolation of new compounds **1**—**7** in addition to the six steroidal saponins reported earlier.[2]

Steroidal nature of **1**—**7** was shown by a positive coloration in Liebermann–Burchard reaction.

Compound **1** was obtained as a white amorphous powder and the molecular formula, $C_{38}H_{62}O_{13}$, was determined by the secondary ion (SI) mass spectrum (MS) which showed quasimolecular ion peaks at *m/z* 749 $[M+Na]^+$ and 727 $[M+H]^+$, and elemental analysis. The infrared (IR) spectrum featured a strong absorption at 3410 cm^{-1} due to hydroxyl group(s), and characteristic absorptions at 975, 915, 895 and 860 cm^{-1} with the absorption at 895 cm^{-1} being of greater intensity than at 915 cm^{-1}, suggesting the presence of the (25*R*)-spiroketal moiety in the molecule.[3]

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed two three-proton singlet signals at δ 0.84 and 0.80 indicating the presence of two angular methyl groups, two three-proton doublet signals at δ 1.13 (J=6.9 Hz) and 0.72 (J=5.3 Hz) attributed to secondary methyl groups, and two one-proton doublet signals at δ 5.27 (J=7.6 Hz) and 4.92 (J=7.9 Hz) assignable to anomeric protons of pyranoses. Hydrolysis of **1** with 1 N hydrochloric acid (H_2O–dioxane, 1 : 1) gave (25*R*)-5α-spirostan-3β,6α-diol (chlorogenin), and D-glucose and D-xylose. The absolute configurations of the sugars were confirmed by the HPLC analysis of the 1-(*N*-acetyl-L-α-methylbenzylamino)-1-deoxyalditol acetate derivatives of the sugars.[4] The presence of a terminal xylose was suggested by the fragment ion peak at *m/z* 595 $[M+H-132]^+$ in the SI-MS of **1**. In the ^{13}C-NMR spectrum of **1**, signals for the aglycon C-6 position and the inner glucose C-3 position were shifted to downfield by *O*-glycosylation as compared with those of chlorogenin and methyl β-D-glucopyranoside, respectively (Table I). The disaccharide with the sequence, β-D-xylopyranosyl-(1→3)-β-D-glucopyranose, was unequivocally concluded to attach to the C-6 hydroxyl position of chlorogenin. Thus, the structure of **1** was formulated as (25*R*)-5α-spirostan-3β,6α-diol (chlorogenin) 6-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucopyranoside.

The molecular formulas of **2**—**4** were determined to be

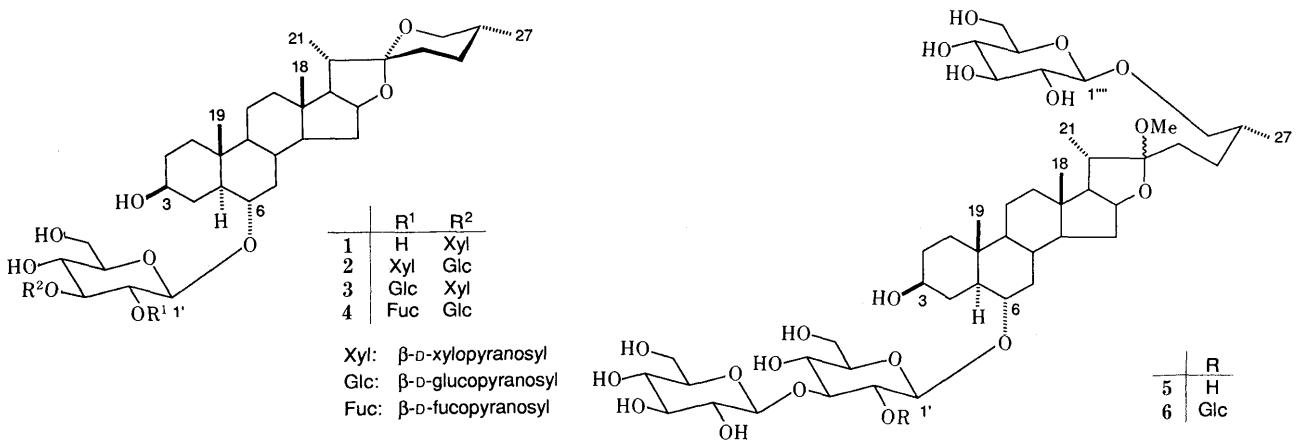
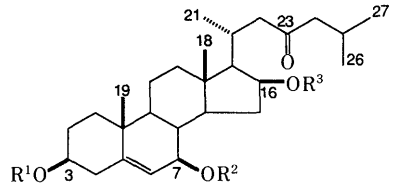


Chart 1



	R^1	R^2	R^3
7	Glc	H	Glc
7a	Glc-(Ac)$_4$	Ac	Glc-(Ac)$_4$
7b	H	H	Glc

Chart 2

TABLE I. ^{13}C-NMR Spectral Data for **1**, Chlorogenin, **2**—**7** and **7b**[a]

	1	Chloro-genin	2	3	4	5	6	7	7b
1	37.8	38.1	37.9	38.1	37.9	37.8	37.9	37.2	37.6
2	32.2[b]	32.4[b]	32.6	32.3[b]	31.4	32.2[b]	32.2[b]	30.3	32.7
3	71.0[c]	71.0	71.0[b]	70.9[c]	70.8	70.7	71.2	78.6	71.2
4	33.3	33.8	33.0	33.0	32.9	33.3	32.8	38.9	43.0
5	51.3	52.8	51.3	51.2	50.9	51.4	51.1	141.6	142.6
6	79.9	68.6	80.1	80.4	78.8[b]	79.6	80.2	128.6	128.0
7	41.4	42.9	40.8	40.8	41.3	41.3	40.6	72.6	72.7
8	34.2	34.4	34.1	34.2	34.0	34.1[c]	34.0[c]	40.5	40.6
9	53.9	54.3	54.0	54.1	54.0	54.0	54.0	48.8	49.0
10	36.7	36.6	36.7	36.8	36.6	36.7	36.6	36.9	36.9
11	21.3	21.4	21.3	21.4	21.3	21.2	21.2	21.3	21.4
12	40.1	40.2	40.1	40.2	40.1	40.0	40.0	39.9	40.0
13	40.8	40.9	40.8	40.9	40.8	41.1	41.1	43.1	43.2
14	56.4	56.5	56.5	56.5	56.5	56.3	56.3	54.6	54.7
15	32.1[b]	32.2[b]	32.1	32.2[b]	32.0	32.0[b]	32.0[b]	38.9	38.9
16	81.0	81.1	81.1	81.2	81.0	81.3	81.3	82.9	82.9
17	63.0	63.1	63.0	63.2	63.0	64.2	64.2	60.9	61.0
18	16.7	16.7	16.7	16.8	16.7	16.2	16.2	13.3	13.3
19	13.6	13.8	13.6	13.9	13.8	13.6	13.7	19.0	19.2
20	42.0	42.0	42.0	42.2	42.0	40.5	40.5	27.5	27.5
21	15.0	15.0	15.0	15.2	15.0	16.6	16.6	19.6	19.6
22	109.1	109.2	109.2	109.3	109.2	112.6	112.6	50.4	50.4
23	31.9	31.8	31.9	32.0	31.8	30.7	30.7	211.6	211.6
24	29.3	29.3	29.3	29.4	29.3	28.3	28.3	52.4	52.4
25	30.6	30.6	30.6	30.8	30.7	34.2[c]	34.2[c]	24.5	24.5
26	66.9	66.9	66.9	67.0	66.8	75.2	75.2	22.8	22.8
27	17.4	17.3	17.3	17.5	17.4[c]	17.2	17.2	22.8	22.8
OMe						47.3	47.3		
1′	105.6		103.5	103.7[d]	104.0	105.4	103.5[d]	102.7	
2′	74.8		79.2	79.9	82.0	74.6	79.9	75.4	
3′	87.9		88.7	88.0	89.8	89.1	89.2	78.4[b]	
4′	69.6		70.1	69.8	70.1	69.9	70.1	71.8	
5′	77.8		77.7	77.8	77.5	77.7	77.6	78.1[b]	
6′	62.5		62.5[c]	62.9[e]	62.5[d]	62.5[d]	62.7[e]	62.9	
1″	106.5		104.9[d]	104.0[d]	104.8[e]	106.1	104.0[d]	106.7	106.8
2″	75.3		75.4	76.4	75.6[f]	75.7	76.1[f]	75.6	75.6
3″	78.2		78.6[e]	78.8[f]	78.7[b]	78.7[e]	78.6[g]	78.8[c]	78.8[b]
4″	70.7[c]		71.2[b]	71.9	71.6[g]	71.7[f]	71.7[h]	72.0	72.0
5″	67.4		67.4	77.8[f]	78.7[b]	78.3[e]	78.6[g]	78.0[c]	78.0[b]
6″				62.6[e]	62.4[d]	62.6[d]	62.5[e]	63.3	63.4
1‴			105.1[d]	105.3	104.9[e]		104.7[d]		
2‴			75.9	75.4	73.1		75.5[f]		
3‴			78.7[e]	78.8[f]	75.7[f]		78.6[g]		
4‴			71.7	71.3[c]	73.1		71.7[h]		
5‴			78.5[e]	67.5	71.4[g]		77.6[g]		
6‴			62.4[c]		17.3[c]		62.4[e]		
1⁗						105.0	104.9		
2⁗						75.2	75.2		
3⁗						78.7[e]	78.6[g]		
4⁗						71.8[f]	71.8[h]		
5⁗						78.5[e]	78.5[g]		
6⁗						63.0	62.9		

a) Spectra were measured in C_5D_5N. *b*—*h*) Assignments with the same superscripts may be reversed in each vertical column.

$C_{44}H_{72}O_{18}$, $C_{44}H_{72}O_{18}$ and $C_{45}H_{74}O_{18}$, respectively, by the SI-MS and elemental analysis. On acid hydrolysis, **2** and **3** yielded chlorogenin, D-glucose and D-xylose, and **4** yielded chlorogenin, D-glucose and D-fucose. In the ^{1}H- and ^{13}C-NMR spectra of **2**—**4**, the signals arising from each aglycon moiety were superimposable on those of **1**. Thus, the fundamental structures of **2**—**4** were proved to be chlorogenin 6-*O*-trisaccharides. The glycosylation shifts in the ^{13}C-NMR spectra of **2**—**4** clearly indicated the presence of the 2,3-linked inner glucopyranside in the molecules, leading to the following possible structures of the trisaccharides: D-xylosyl-(1→2)-*O*-[D-glucosyl-(1→3)]-D-glucoside or D-glucosyl-(1→2)-*O*-[D-xylosyl-(1→3)]-D-glucoside in **2** and **3**, and D-fucosyl-(1→2)-*O*-[D-glucosyl-(1→3)]-D-glucoside or D-glucosyl-(1→2)-*O*-[D-fucosyl-(1→3)]-D-glucoside in **4** (Table I). The configuration of the anomeric center of each pyranose was assigned as the *β*-form from its coupling constant (J=6.7—8.1 Hz) in the ^{1}H-NMR spectrum. Partial hydrolysis allowed establishment of the complete structures of the oligoglycoside moieties. Hydrolysis of **2** and **4** under a mild condition gave chlorogenin 6-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranoside,[2] and **3** gave chlorogenin 6-*O*-*β*-D-glcopyranosyl-(1→2)-*β*-D-glucopyranoside.[2] Accordingly, the full structures of **2**—**4** were characterized as chlorogenin 6-*O*-*β*-D-xylopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside, chlorogenin 6-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranoside and chlorogenin 6-*O*-*β*-D-fucopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside.

Compounds **5**, $C_{46}H_{78}O_{20}$ and **6**, $C_{52}H_{88}O_{25}$, were suggested to be furostanol glycosides by Ehrlich's test[5] and the IR spectra. The ^{1}H-NMR spectrum of **5** showed signals for two tertiary methyl groups at δ 0.85 and 0.76, two secondary methyl groups at δ 1.17 (J=6.8 Hz) and 1.02 (J=6.6 Hz), a methoxyl group at δ 3.24, and three anomeric protons at δ 5.28 (J=7.8 Hz), 4.92 (J=7.6 Hz) and 4.84 (J=7.7 Hz). Enzymatic hydrolysis with *β*-glucosidase in acetic acid/sodium acetate buffer (pH 5) gave D-glucose and spirostanol glycosides, the structures of which were identified as chlorogenin 6-*O*-*β*-D-glucopyranoside and chlorogenin 6-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranoside by the IR and ^{1}H-NMR spectra.[2] Thus, the structure of **5** was confirmed to be 22-*O*-methyl-26-*O*-*β*-D-glucopyranosyl-(25*R*)-5*α*-furostan-3*β*,6*α*,22*ξ*-triol 6-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranoside. The signals due to the aglycon moiety of **6** were in good agreement with those of **5** in the ^{1}H- and ^{13}C-NMR spectra. The presence of one more terminal glucose was easily recognized by the ^{13}C-NMR spectrum (Table I). Enzymatic hydrolysis with *β*-glucosidase furnished D-glucose and the corresponding spirostanol glycoside, identified as chlorogenin 6-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside by the IR and ^{1}H-NMR spectra.[2] The structure of **6** was determined to be 22-*O*-methyl-26-*O*-*β*-D-glucopyranosyl-(25*R*)-5*α*-furostan-3*β*,6*α*,22*ξ*-triol 6-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranoside.

Compound **7**, $C_{39}H_{64}O_{14}$, was distinctive in showing a characteristic bright blue spot on thin layer chromatography (TLC) when sprayed with 10% sulphuric acid and by heating. The IR spectrum indicated the existence of hydroxyl group(s) (3420 cm^{-1}) and an carbonyl group (1695 cm^{-1}). The ^{1}H-NMR spectrum showed signals for two tertiary methyl groups at δ 0.96 and 0.89, three secondary methyl

groups at δ 1.12 (J=6.6 Hz), 0.92 (J=5.9 Hz) and 0.90 (J=6.5 Hz), and two anomeric protons at δ 5.04 (J=7.7 Hz) and 4.73 (J=7.7 Hz). The ^{13}C-NMR spectrum showed 12 signals due to two terminal β-glucose moieties and 27 signals due to the aglycon moiety. The 27 carbons were readily separated to $CH_3 \times 5$, $CH_2 \times 8$, $CH \times 10$ and $C \times 4$ with the help of the various distortionless enhancement by polarization transfer (DEPT) spectra. The signals at δ 211.6 (C), 141.6 (C) and 128.6 (CH), and 82.9 (CH), 78.6 (CH) and 72.6 (CH) were respectively assigned to a carbonyl group, a double bond and the carbons bearing oxygen functions. Acetylation of **7** with acetic anhydride in pyridine gave the corresponding nonaacetate (**7a**). The data presented above suggested that **7** was cholestene derivative with a carbonyl group, a hydroxyl group and two hydroxyl groups bearing β-D-glucopyranose. On acid hydrolysis of **7**, the aglycon moiety was decomposed to yield several unidentified artifactual compounds, and on enzymatic hydrolysis with β-glucosidase, **7** was recovered unchanged after 72 h incubation. Enzymatic hydrolysis using hesperidinase cleaved one of the two β-D-glucosyl groups attached to the aglycon to yield a monoglucoside (**7b**). On comparison of the ^{1}H and ^{13}C chemical shifts of the A and B rings of **7b** with those of cholesterol,[6] the presence of the C-7 hydroxyl group in addition to the 3β-hydroxyl-5-ene group was suggested. The ^{13}C-NMR spectrum is available to differentiate the orientations of the C-7 hydroxyl isomers of cholest-5-ene-3β,7-diol.[7] The ^{13}C signals of **7b** agreed with those of cholest-5-ene-3β,7β-diol.[6] Next, by tracing out the ^{1}H–^{1}H coupling network from the terminal secondary methyl groups (H_3-21, H_3-26 and H_3-27) through double resonance experiments, the presence of the C-23 carbonyl group and the C-16 hydroxyl or glucosyloxy group was revealed (Fig. 1). The ^{1}H-NMR chemical shift of the H-18 methyl group (δ 0.99) (that of cholesterol appeared at δ 0.70) proved the orientation of the C-16 substituted group to be β.[7] Thus, the structure of the aglycon moiety was shown to be 3β,7β,16β-trihydroxycholest-5-en-23-one. The substituted positions of β-D-glucopyranose were confirmed to be the C-3 and C-16 hydroxyl positions, since, in the ^{1}H-NMR spectrum of **7a**, the signal for the H-7 methine proton was shifted to a lower field by O-acetylation

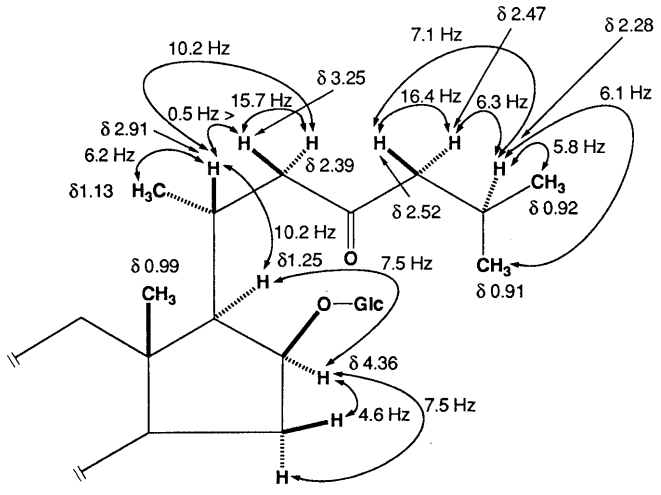


Fig. 1. ^{1}H-NMR Chemical Shifts and Coupling Network of the D-Ring and Side Chain of **7b** (C_5D_5N)

to appear at δ 4.96 (br d, J=7.9 Hz), whereas the signals for the H-3 and H-16 methine protons remained unaffected [H-3: δ 3.48 (m); H-16: δ 3.97 ddd (J=7.3, 7.3, 3.9 Hz)]. Finally, the structure of **7** was determined to be 3,16-bis-O-β-D-glucopyranoside of 3β,7β,16β-trihydroxycholest-5-en-23-one, and it was designated as camassioside.

Chlorogenin glycosides and a polyhydroxylated cholestane bisdesmoside such as camassioside are rare in nature.[2,8] Biological tests of the compounds isolated in this study are in progress.

Experimental

The following instruments were used for measurements of the spectral and physical data. Optical rotations were measured with a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 instrument, and MS on a Hitachi M-80 machine. NMR spectra were taken with a Bruker AM-400 spectrometer. Chemical shifts are given in δ-values referring to internal tetramethylsilane (TMS), and the following abbreviations are used; s=singlet, d=doublet, dd=doublet of doublets, m=multiplet, br=broad. Fuji Davison silica gel (BW-300, Fuji Davison Co., Ltd.) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Inc.) were used for column chromatographies. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck), and spots were visualized by spraying 10% H_2SO_4 followed by heating. HPLC was performed with a Tosoh HPLC system (Tosoh Co., Ltd.: pump, Tosoh CCPM; controller, CCP controller PX-8010; detector, Tosoh RI-8010 or Tosoh UV-8000) equipped with a CIG pre-packed column (Kusano Kagakukikai Co., Ltd. 20 mm i.d. × 100 mm, ODS, 20 μm) for the preparative HPLC or with a Kaseisorb LC ODS-120-5 column (Tokyo Kasei Kogyo Co., Ltd. 4.6 mm i.d. × 250 mm, ODS, 5 μm) for the analytical HPLC, or the HPLC system (pump, Tosoh CCPP-M; controller, CCP controller PX-8010; detector, ERC-7530 (ERMA Inc.)) equipped with a Kaseisorb LC ODS-120-5 column (20 mm i.d. × 250 mm, ODS, 5 μm).

Isolation The n-BuOH soluble phase of the methanolic extract of the bulbs of *Camassia cusickii* (4.4 kg) was fractionated on a silica gel column with a CH_2Cl_2–MeOH gradient system to six fractions as described previously.[2] Further fractionation on a silica gel column with $CHCl_3$–MeOH–H_2O system, an ODS column with MeOH–H_2O system and HPLC with MeOH–H_2O system led to the isolation of **1** from fraction 3, **2**, **3**, **4** and **7** from fraction 4, and **5** and **6** from fraction 5.

Chlorogenin 6-O-β-D-Xylopyranosyl-(1→3)-β-D-glucopyranoside (1) A white amorphous powder (18.4 mg), $[\alpha]_D^{25}$ −15.6° (c=0.27, MeOH). SI-MS m/z: 749 $[M+Na]^+$, 727 $[M+H]^+$, 595 $[M-\text{pentose}+H_2O+H)]^+$, 433 $[\text{aglycon}+H]^+$, 415 $[\text{aglycon}-OH]^+$. *Anal.* Calcd for $C_{38}H_{62}O_{13}$: C, 62.79; H, 8.60. Found: C, 62.25; H, 8.87. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2930, 2860 (CH), 1445, 1370, 1235, 1165, 1150, 1075, 1035, 975, 950, 915, 895, 860 ((25R)-spiroacetal, intensity 915<895). ^{1}H-NMR (C_5D_5N) δ: 5.27 (1H, d, J=7.6 Hz, H-1″), 4.92 (1H, d, J=7.9 Hz, H-1′), 3.80 (1H, m, H-3), 3.72 (1H, ddd, J=9.8, 9.8, 3.5 Hz, H-6), 3.57 (1H, dd, J=10.5, 2.7 Hz, H-26a), 3.47 (1H, dd, J=10.5, 10.5 Hz, H-26b), 1.13 (3H, d, J=6.9 Hz, H-21), 0.84 (3H, s, H-19), 0.80 (3H, s, H-18), 0.72 (3H, d, J=5.3 Hz, H-27).

Chlorogenin 6-O-β-D-Xylopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (2) A white amorphous powder (18.9 mg), $[\alpha]_D^{24}$ −24.4° (c=0.83, MeOH). SI-MS m/z: 926 $[M+K-H]^+$, 912 $[M+Na+H]^+$, 889 $[M+H]^+$. *Anal.* Calcd for $C_{44}H_{72}O_{18}\cdot 2H_2O$: C, 57.13; H, 8.28. Found: C, 57.66; H, 8.21. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2920, 2865 (CH), 1440, 1370, 1295, 1235, 1150, 1070, 1040, 975, 950, 910, 890, 855 ((25R)-spiroacetal, intensity 910<890). ^{1}H-NMR (C_5D_5N) δ: 5.55 (1H, d, J=6.7 Hz, H-1″), 5.29 (1H, d, J=8.1 Hz, H-1‴), 4.87 (1H, d, J=8.0 Hz, H-1′), 3.90 (1H, m, H-3), 3.72 (1H, ddd, J=10.3, 10.3, 4.5 Hz, H-6), 3.56 (1H, dd, J=10.4, 2.7 Hz, H-26a), 3.46 (1H, dd, J=10.4, 10.4 Hz, H-26b), 1.12 (3H, d, J=6.9 Hz, H-21), 0.83 (3H, s, H-19), 0.79 (3H, s, H-18), 0.71 (3H, d, J=5.1 Hz, H-27).

Chlorogenin 6-O-β-D-Glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranoside (3) A white amorphous powder (486 mg), $[\alpha]_D^{25}$ −19.4° (c=0.33, $CHCl_3$–MeOH (1:3)). SI-MS m/z: 911 $[M+Na]^+$. *Anal.* Calcd for $C_{44}H_{72}O_{18}\cdot 2H_2O$: C, 57.13; H, 8.28. Found: C, 56.89; H, 8.34. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2940, 2875 (CH), 1450, 1375, 1240, 1155, 1075, 1035, 980, 915, 895, 860 ((25R)-spiroacetal, intensity 915<895). ^{1}H-NMR (C_5D_5N) δ: 5.76 (1H, d, J=7.7 Hz, H-1″ or H-1‴), 5.36 (1H, d, J=7.7 Hz, H-1″ or H-1‴), 4.85 (1H, d, J=7.6 Hz, H-1′), 3.91 (1H, m, H-3), 3.66 (1H, ddd, J=10.4, 10.4, 4.3 Hz, H-6), 3.57 (1H, dd, J=10.4,

2.3 Hz, H-26a), 3.47 (1H, dd, J=10.4, 10.4 Hz, H-26b), 1.13 (3H, d, J=6.9 Hz, H-21), 0.83 (3H, s, H-19), 0.81 (3H, s, H-18), 0.72 (3H, d, J=5.1 Hz, H-27).

Chlorogenin 6-*O*-β-D-Fucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (4) A white amorphous powder (38.5 mg), $[\alpha]_D^{25}$ +1.9° (c=0.63, MeOH). SI-MS m/z: 925 $[M+Na]^+$. *Anal.* Calcd for $C_{45}H_{74}O_{18}\cdot 2H_2O$: C, 57.55; H, 8.37. Found: C, 56.98; H, 8.27. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2940 (CH), 1450, 1375, 1240, 1170, 1155, 1070, 1055, 1030, 980, 955, 915, 895, 860 ((25*R*)-spiroacetal, intensity 915<899). ^{1}H-NMR (C_5D_5N) δ: 5.60 (1H, d, J=7.8 Hz, H-1″ or H-1‴), 5.35 (1H, d, J=7.8 Hz, H-1″ or H-1‴), 4.76 (1H, d, J=7.6 Hz, H-1′), 3.68 (1H, m, H-3), 3.59 (1H, dd, J=10.5, 2.8 Hz, H-26a), 3.49 (1H, m, H-6), 3.48 (1H, dd, J=10.5, 10.5 Hz, H-26b), 1.55 (3H, d, J=6.4 Hz, H-6″), 1.12 (3H, d, J=6.9 Hz, H-21), 0.87 (3H, s, H-19), 0.81 (3H, s, H-18), 0.73 (3H, d, J=5.2 Hz, H-27).

Acid Hydrolysis of 1—4 and Determination of the Absolute Configurations of Sugars A solution of each saponin (3.5 mg) in 1 N HCl (H_2O–dioxane, 1:1) (2 ml) was heated in a sealed tube for 1 h at 100 °C. After cooling, the reaction mixture was neutralized with an Amberlite IRA-93ZU (OH^- form) (Organo Co., Ltd.) column. A Sep-Pak C_{18} cartridge (Waters) was applied to fractionate the reaction mixture into the sugar fraction using H_2O–MeOH (9:1) as the eluent and into the sapogenin fraction using H_2O–MeOH (1:9). The sapogenin constituting **1**—**4** was identified as chlorogenin by direct TLC comparison with an authentic sample (*Rf* 0.31, $CHCl_3$–MeOH (15:1); *Rf* 0.25, $CHCl_3$–Me_2CO (3:1)). Each sugar fraction was treated with L-(−)-α-methylbenzylamine (7 mg) and $NaBH_3CN$ (2 mg) for 3 h at 40 °C, followed by acetylation with Ac_2O in pyridine containing a catalytic amount of 4-(dimethylamino)pyridine. The 1-(*N*-acetyl-L-α-methylbenzylamino)-1-deoxyalditol acetates of the monosaccharides were analyzed under the following condition: column, Kaseisorb LC ODS-120-5 (4.6 mm i.d. × 250 mm, ODS, 5 μm); solvent, MeCN–H_2O (2:3); flow rate, 0.8 ml/min; detection, ultraviolet (UV) (230 nm). The derivatives of D-glucose and D-xylose were detected in the sugar fractions of **1**, **2** and **3**, and the derivatives of D-glucose and D-fucose in the sugar fraction of **4**. t_R (min): L-xylose, 19.6; D-xylose, 20.7; D-fucose, 24.4; L-fucose, 26.4; L-glucose, 26.6; D-glucose, 28.3.

Partial Hydrolysis of 2—4 A solution of each saponin (2.0 mg) in 0.2 N HCl (dioxane–H_2O, 1:1) (2 ml) was heated in a sealed tube for 30 min at 100 °C. The reaction mixture was neutralized with an Amberlite IRA-93ZU (OH^- form) column, and passed through a Sep-Pak C_{18} cartridge with H_2O–MeOH (9:1) and then with H_2O–MeOH (1:9). The H_2O–MeOH (1:9) eluate was analyzed by HPLC: column, Kaseisorb LC ODS-120-5 (4.6 mm i.d. × 250 mm, ODS, 5 μm); flow rate: 0.55 m/min; detection, refractive index (RI). Chlorogenin 6-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (t_R 12.5) was detected in the reaction mixtures of **2** and **4**, and chlorogenin 6-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (t_R 11.6) in the reaction mixture of **3**.

22-*O*-Methyl-26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-3β,6α,22ξ-triol 6-*O*-β-D-Glucopyranosyl-(1→3)-β-D-glucopyranoside (5) A white amorphous powder (100 mg), $[\alpha]_D^{24}$ −21.8° (c=0.33, MeOH). SI-MS m/z: 919 $[M-OMe]^+$. *Anal.* Calcd for $C_{46}H_{78}O_{20}\cdot 2H_2O$: C, 55.46; H, 8.40. Found: C, 55.06; H, 8.07. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2925 (CH), 1450, 1375, 1260, 1155, 1070, 1025, 890. ^{1}H-NMR (C_5D_5N) δ: 5.28 (1H, d, J=7.8 Hz, H-1″), 4.92 (1H, d, J=7.6 Hz, H-1⁗), 4.84 (1H, d, J=7.7 Hz, H-1′), 3.80 (1H, m, H-3), 3.74 (1H, ddd, J=10.7, 10.7, 4.5 Hz, H-6), 3.24 (3H, s, OMe), 1.17 (3H, d, J=6.8 Hz, H-21), 1.02 (3H, d, J=6.6 Hz, H-27), 0.85 (3H, s, H-19), 0.76 (3H, s, H-18).

22-*O*-Methyl-26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-3β,6α,22ξ-triol 6-*O*-β-D-Glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (6) A white amorphous powder (177 mg), $[\alpha]_D^{24}$ −18.7° (c=0.31, MeOH). SI-MS m/z: 1081 $[M-OMe]^+$. *Anal.* Calcd for $C_{52}H_{88}O_{25}$: C, 56.10; H, 7.97. Found: C, 56.95; H, 8.28. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2925 (CH), 1445, 1375, 1250, 1195, 1155, 1070, 1025, 890. ^{1}H-NMR (C_5D_5N) δ: 5.74 (1H, d, J=7.6 Hz, H-1″ or H-1‴), 5.38 (1H, d, J=7.8 Hz, H-1″ or H-1‴), 4.92 (H-1⁗, overlapping with H_2O signal), 4.84 (1H, d, J=7.6 Hz, H-1′), 3.90 (1H, m, H-3), 3.67 (1H, ddd, J=10.5, 10.5, 4.2 H, H-6), 3.24 (3H, s, OMe), 1.17 (3H, d, J=6.9 Hz, H-21), 1.01 (3H, d, J=6.5 Hz, H-27), 0.83, (3H, s, H-19), 0.78 (3H, s, H-18).

Enzymatic Hydrolysis of 5 and 6 Compound **5** (30.0 mg) was treated with β-glucosidase (Tokyo Kasei Co., Ltd.) (15 mg) in the AcOH/AcONa buffer (pH 5) at room temperature. The reaction mixture was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (100:20:1→60:20:1) to furnish D-glucose (5.4 mg), chlorogenin 6-*O*-β-D-glucopyranoside (4.7 mg) and chlorogenin 6-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (16.5 mg). Compound **6** (30.0 mg) was treated as in the case of **5** and the reaction mixture was chromatographed on silica gel with (80:20:1→40:20:1) and ODS with MeOH–H_2O (4:1) to furnish D-glucose (4.4 mg) and chlorogenin 6-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (17.5 mg).

3β,7β,16β-Trihydroxycholest-5-en-23-one 3,16-Bis-*O*-β-D-glucopyranoside (Camassioside) (7) A white amorphous powder (21.9 mg), $[\alpha]_D^{28}$ −5.0° (c=0.52, MeOH). SI-MS m/z: 795 $[M+K]^+$, 779 $[M+Na]^+$, 778 $[M+Na-H]^+$. *Anal.* Calcd for $C_{39}H_{64}O_{14}\cdot H_2O$: C, 60.45; H, 8.58. Found: C, 59.84; H, 8.49. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2940 (CH), 1695 (C=O), 1460, 1365, 1305, 1255, 1200, 1160, 1075, 1030, 890. ^{1}H-NMR (C_5D_5N) δ: 5.64 (1H, br s, H-6), 5.04 (1H, d, J=7.7 Hz, H-1″), 4.73 (1H, d, J=7.7 Hz, H-1′), 4.35 (1H, ddd, J=7.9, 7.9, 4.5 Hz, H-16), 3.23 (1H, br d, J=14.4 Hz, H-22a), 2.90 (1H, m, H-20), 2.76 (1H, dd, J=13.3, 2.6 Hz, H-4 equatorial), 2.52 (1H, dd, J=16.4, 7.0 Hz, H-24a), 2.47 (1H, dd, J=16.4, 6.7 Hz, H-24b), 2.46 (1H, dd, J=13.3, 13.3 Hz, H-4 axial), 2.39 (1H, dd, J=14.4, 10.3 Hz, H-22b), 2.27 (1H, m, H-25), 1.24 (1H, dd, J=10.8, 7.9 Hz, H-17), 1.12 (3H, d, J=6.6 Hz, H-21), 0.96 (3H, s, H-19), 0.92 (3H, d, J=5.9 Hz, H-26 or H-27), 0.90 (3H, d, J=6.5 Hz, H-26 or H-27), 0.89 (3H, s, H-18).

Acetylation of 7 Compound **7** (4.0 mg) was acetylated with Ac_2O in pyridine containing a catalytic amount of 4-(dimethylamino)pyridine and the crude acetate was chromatographed on silica gel with *n*-hexane–Me_2CO (5:2) to yield the peracetate (**7a**) (5.1 mg) as a white amorphous powder. $[\alpha]_D^{24}$ +13.2° (c=0.28, MeOH). IR ν_{max}^{KBr} cm^{-1}: 2945 (CH), 1755 (C=O), 1430, 1365, 1220, 1170, 1135, 1030, 950, 900. ^{1}H-NMR (C_5D_5N) δ: 5.20 (1H, br s, H-6), 5.19 (1H, dd, J=9.5, 9.5 Hz, H-3′ or H-3″), 5.13 (1H, dd, J=9.5, 9.5 Hz, H-3′ or H-3″), 5.06 (1H, dd, J=9.5, 9.5 Hz, H-4′ or H-4″), 4.99 (1H, dd, J=9.5, 9.5 Hz, H-4′ or H-4″), 4.98 (1H, dd, J=9.5, 7.8 Hz, H-2′ or H-2″), 4.96 (1H, br d, J=7.9 Hz, H-7), 4.94 (1H, dd, J=9.5, 8.0 Hz, H-2′ or H-2″), 4.56 (1H, d, J=8.0 Hz, H-1′ or H-1″), 4.38 (1H, d, J=7.8 Hz, H-1′ or H-1″), 4.24 (1H, dd, J=12.3, 4.8 Hz, H-6′a or H-6″a), 4.18 (1H, dd, J=12.3, 5.7 Hz, H-6′a or H-6″a), 4.12 (1H, dd, J=12.3, 2.4 Hz, H-6′b or H-6″b), 4.04 (1H, dd, J=12.3, 2.3 Hz, H-6′b or H-6″b), 3.97 (1H, ddd, J=7.3, 7.3, 3.9 Hz, H-16), 3.66 (2H, overlapping, H-5′ and H-5″), 3.48 (1H, m, H-3), 2.08, 2.07, 2.05, 2.03, 2.02, 2.01, 1.99, 1.98 × 2 (each 3H, s, Ac), 1.05 (3H, s, H-19), 0.97 (3H, d, J=6.2 Hz, H-21), 0.93 (3H, d, J=6.6 Hz, H-26 or H-27), 0.92 (3H, d, J=6.6 Hz, H-26 or H-27), 0.87 (3H, s, H-18).

Enzymatic Hydrolysis of 7 A mixture of **7** (10.0 mg) and hesperidinase (Sigma Chemical Co.) (50 mg) in AcOH/AcOK buffer (pH 4.3) was incubated at room temperature for 72 h. The reaction mixture was chromatographed on a silica gel column using $CHCl_3$–MeOH (7:1) and $CHCl_3$–MeOH–H_2O (20:10:1) to yield D-glucose (1.8 mg) and 3-desglucosyl derivative (**7b**) (6.0 mg) as a white amorphous powder. $[\alpha]_D^{24}$ +4.3° (c=0.28, MeOH). SI-MS m/z: 595 $[M+H]^+$, 431 $[aglycon-H]^+$, 415 $[aglycon-OH]^+$, 397 $[aglycon-H_2O-OH]^+$. IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2935 (CH), 1695 (C=O), 1460, 1370, 1295, 1260, 1075, 1040, 950, 895. ^{1}H-NMR (C_5D_5N) δ: 5.72 (1H, br s, H-6), 4.73 (1H, d, J=7.8 Hz, H-1′), 4.36 (1H, ddd, J=7.5, 7.5, 4.6 Hz, H-16), 3.25 (1H, br d, J=15.7 Hz, H-22a), 2.91 (1H, m, H-20), 2.68 (1H, br d, J=11.5 Hz, H-4 equatorial), 2.61 (1H, dd, J=11.5, 11.5 Hz, H-4 axial), 2.52 (1H, dd, J=16.4, 7.1 Hz, H-24a), 2.47 (1H, dd, J=16.4, 6.3 Hz, H-24b), 2.39 (1H, dd, J=15.7, 10.2 Hz, H-22b), 2.28 (1H, m, H-25), 1.25 (1H, dd, J=10.2, 7.5 Hz, H-17), 1.13 (3H, d, J=6.2 Hz, H-21), 1.03 (3H, s, H-19), 0.99 (3H, s, H-18), 0.92 (3H, d, J=5.8 Hz, H-26 or H-27), 0.91 (3H, d, J=6.1 Hz, H-26 or H-27).

Acknowledgements We wish to thank Dr. Y. Shida and Mrs. Y. Katoh of the Central Analytical Center of our college for the measurements of MS.

References and Notes

1) S. B. Mahato, A. N. Ganguly and N. P. Sahu, *Phytochemistry*, **21**, 959 (1982).
2) Y. Mimaki, Y. Sashida and K. Kawashima, *Phytochemistry*, **30**, 3721 (1991).
3) *a*) M. E. Wall, C. R. Eddy, M. L. McClennan and M. E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952); *b*) C. R. Eddy, M. E. Wall and M. K. Scott, *ibid.*, **25**, 266 (1953); *c*) R. N. Jones, K. Katzenellenbogen and K. Dobriner, *J. Am. Chem. Soc.*, **75**, 158 (1953).
4) *a*) R. Oshima and J. Kumanotani, *Chem. Lett.*, **1981**, 943; *b*) R. Oshima, Y. Yamauchi and J. Kumanotani, *Carbohydr. Res.*, **107**, 169 (1982); *c*) Y. Asada, M. Ikeno, T. Ueoka and T. Furuya, *Chem. Pharm. Bull.*, **37**, 2747 (1989); *d*) B.-Y. Yu, Y. Hirai, J. Shoji and G.-J. Xu, *ibid.*, **38**, 1931 (1990).

5) *a*) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa and T. Kawasaki, *Chem. Pharm. Bull.*, **16**, 1162 (1968); *b*) T. Nohara, K. Miyahara and T. Kawasaki, *ibid.*, **23**, 872 (1975).
6) ^{1}H-NMR of cholesterol (C_5D_5N) δ: 5.43 (1H, br d, J=4.7 Hz, H-6), 3.82 (1H, m, H-3), 1.08 (3H, s, H-19), 0.99 (3H, d, J=6.3 Hz, H-21), 0.90 (6H, d, J=6.6 Hz, H-27), 0.70 (3H, s, H-18). ^{13}C-NMR of cholesterol (C_5D_5N) δ: 37.9, 32.3, 71.3, 43.5, 142.0, 121.2, 32.7, 32.2, 50.6, 37.0, 21.4, 40.1, 42.6, 57.0, 24.6, 28.5, 56.5, 12.2, 19.7, 36.1, 19.0, 36.6, 24.2, 39.8, 28.3, 23.0, 22.7 (C-1—C-27).
7) M. Kobayashi, F. Kanda, S. R. Damarla, D. V. Rao and C. B. Rao, *Chem. Pharm. Bull.*, **38**, 2400 (1990).
8) *a*) J. Kitajima, T. Komori and T. Kawasaki, *Yakugaku Zasshi*, **102**, 1009 (1982); *b*) S. C. Sharma and O. P. Sati, *Phytochemistry*, **21**, 1820 (1982).