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Steroidal glycosides from the bulbs of *Lilium speciosum*

Tomoki Iguchi*, Akihito Yokosuka, Minpei Kuroda, Mayuko Takeya, Mizuki Hagiya, Yoshihiro Mimaki

Department of Medicinal Pharmacognosy, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

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ABSTRACT

Steroidal glycosides (**1**-**21**), including eight new spirostanol glycosides (**1**-**8**) and a new cholestane glycoside (**9**), were isolated from the bulbs of *Lilium speciosum*. The structures of the new compounds were determined based on spectroscopic data analysis and hydrolytic cleavage reactions. The isolated compounds and their aglycones (**1a**, **8a**, and **9a**) were evaluated for their cytotoxicity against HL-60 human acute promyelocytic leukemia cells and A549 human lung carcinoma cells. Compounds **12** and **21** exhibited moderate cytotoxic activity toward HL-60 and A549 cells, with IC_{50} values of 6.9 μM and 5.1 μM against HL-60 cells, and 7.6 μM and 4.4 μM against A549 cells, respectively. Compound **20** only showed moderate cytotoxicity toward A549 cells, with an IC_{50} value of 6.7 μM.

1. Introduction

Plants belonging to the genus *Lilium* are rich sources of phenolic and steroidal glycosides (Mimaki et al., 1989; Mimaki and Sashida, 1990a, b, c; Mimaki et al., 1993, 1994; Satou et al., 1996; Mimaki et al., 1999; Munafo et al., 2010; Hong et al., 2012; Zhou et al., 2012; Matsuo et al., 2015; Thi et al., 2019). Previously, we have performed chemical examinations of the bulbs of *Lilium speciosum* var. *rubrum* and *L. speciosum* froma *vestale*, and isolated a series of phenylpropanoid sucrose esters, a glycerol glucoside, and steroidal glycosides (Shimomura et al., 1986; Mimaki and Sashida, 1991). However, no phytochemical study of the bulbs of *Lilium speciosum* species was conducted. *L. speciosum* is a perennial plant that is mainly distributed in west Japan and is also called the "Japanese lily" (Tsukamoto, 1989). This phytochemical investigation of *L. speciosum* bulbs focused on its steroidal constituents, and resulted in the isolation of 21 steroidal glycosides (**1**-**21**), including eight new spirostanol glycosides (**1**-**8**) and a new cholestane glycoside (**9**). The structures of the new compounds were determined by spectroscopic data analysis and hydrolytic cleavage reactions. The isolated compounds (**1**-**21**) and their aglycones (**1a**, **8a**, and **9a**) were evaluated for their cytotoxic activity against HL-60 human acute promyelocytic leukemia cells and A549 human lung carcinoma cells.

2. Results and discussion

L. speciosum bulbs (fresh weight, 8.7 kg) were extracted with hot MeOH. After removing the solvent, the MeOH extract (340 g) was subjected to a Diaion HP-20 porous-polymer polystyrene resin column, and successively eluted with 30 % MeOH, 50 % MeOH, MeOH, EtOH, and EtOAc. The MeOH eluted portion (45 g) was repeatedly fractionated by column chromatography (CC) on silica gel and octadecylsilanized (ODS) silica gel, and by preparative ODS HPLC to obtain 21 steroidal glycosides (**1**-**21**). The structures of the known compounds were identified as (25*R*,26*R*)-17α-hydroxy-26-methoxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**10**) (Mimaki et al., 1998), (25*S*)-17α,27-dihydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**11**) (Ono et al., 2007), (25*R*)-spirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**12**) (Han et al., 1999), (25*R*,26*R*)-26-methoxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**13**) (Mimaki and Sashida, 1991), (25*S*)-27-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**14**) (Mimaki and Sashida, 1990a), (25*S*)-27-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**15**) (Mimaki and Sashida, 1990b), (25*S*)-27-hydroxyspirost-5-en-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**16**) (Mimaki et al., 1998), (25*R*)-27-[((3*S*)-3-hydroxy-3-methylglutaroyl)oxy]-spirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**17**) (Mimaki et al., 1993), (25*R*)-27-[((3*S*)-3-hydroxy-3-methylglutaroyl)oxy]-spirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**18**) (Mimaki et al., 1993), (25*R*)-3-[(*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl)oxy]-5α-spirostan-6-one (**19**) (Matsuo et al., 2013), (25*R*)-26-[(β-D-glucopyranosyl)oxy]-furosta-

* Corresponding author.
E-mail address: iguchit@toyaku.ac.jp (T. Iguchi).

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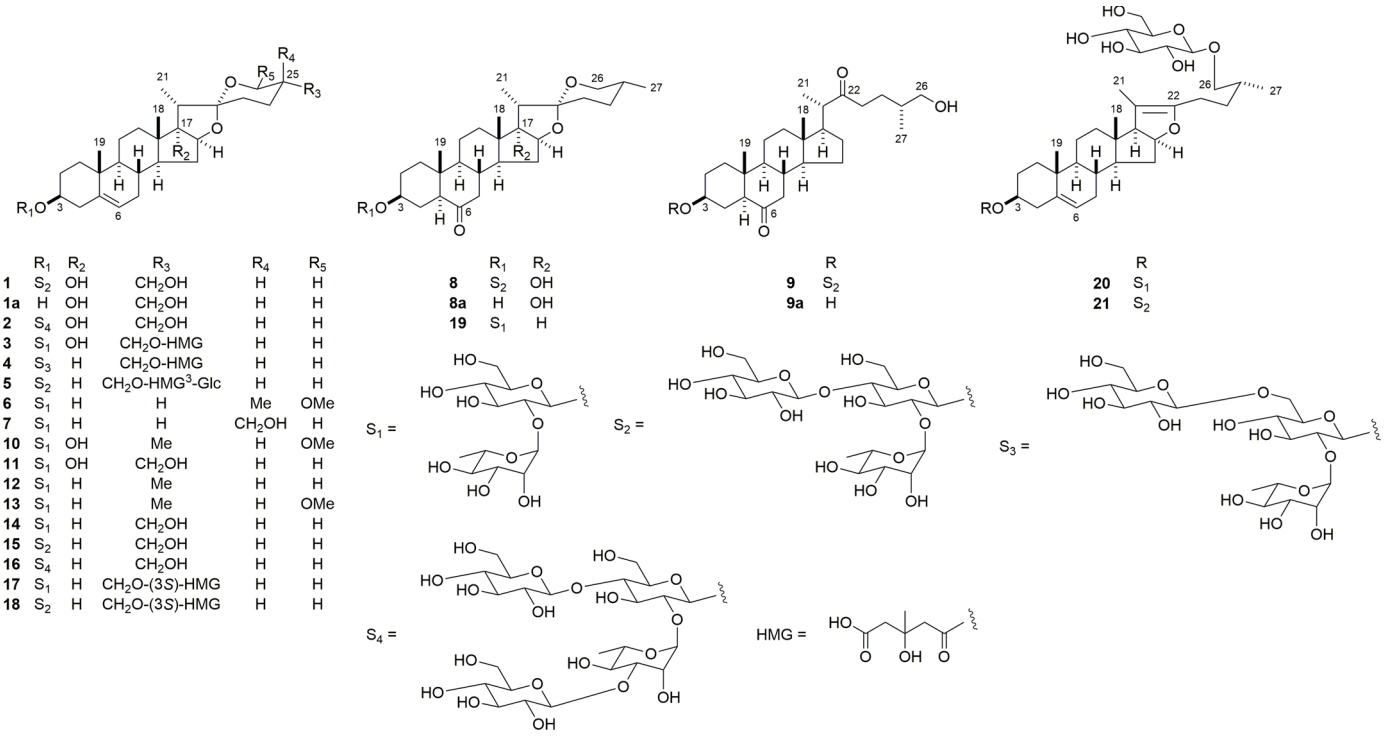

Fig. 1. Structures of **1**, **1a**, **2**-**8**, **8a**, **9**, **9a**, and **10**-**21**.

5,20(22)-dien-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**20**) (Dong et al., 2001), and (25*R*)-26-[(β-D-glucopyranosyl)oxy]-furosta-5,20(22)-dien-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (**21**) (Ali et al., 2013), respectively (Fig. 1).

Compound **1** was isolated as an amorphous powder. Its molecular formula was assigned as $C_{45}H_{72}O_{19}$ based on the high-resolution electrospray ionization time-of-flight mass spectroscopy (HRESITOFMS) and ^{13}C-NMR spectral data. The ^{1}H-NMR spectral features of the aglycone moiety of **1** were closely related to those of **11**, showing signals for two angular methyl groups at δ_H 1.05 (3H, s, Me-19) and 0.95 (3H, s, Me-18), a secondary methyl group at δ_H 1.23 (3H, d, J = 7.1 Hz, Me-21), two oxymethylene groups at δ_H 4.06 (1H, dd, J = 11.3, 4.7 Hz, H-26a) and 3.88 (1H, dd, J = 11.3, 11.3 Hz, H-26b), and δ_H 3.72 (1H, dd, J = 10.6, 5.2 Hz, H-27a) and 3.63 (1H, dd, J = 10.6, 7.4 Hz, H-27b), two oxymethine protons at δ_H 4.47 (1H, dd, J = 7.1, 6.7 Hz, H-16) and 3.82 (1H, m, $W_{1/2}$ = 20.7 Hz, H-3), and an olefinic proton at δ_H 5.27 (1H, br d, J = 4.1 Hz, H-6). Enzymatic hydrolysis of **1** with naringinase afforded (25*S*)-spirost-5-ene-3β,17α,27-triol (**1a**) (Yokosuka and Mimaki, 2008) as the aglycone, and D-glucose and L-rhamnose as the carbohydrate moieties. The ^{1}H-^{1}H correlation spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC) spectra of **1** allowed the sequential assignments of the ^{1}H- and ^{13}C-NMR signals for each sugar unit, indicating that the sugar moiety of **1** comprised a 2,4-disubstituted inner β-D-glucopyranosyl unit [Glc (I): δ_H 4.91 (1H, d, J = 7.6 Hz, H-1′); δ_C 99.9, 77.3, 77.6, 81.9, 76.1, and 61.8 (C-1′-6′)], a terminal α-L-rhamnopyranosyl unit [Rha: δ_H 6.20 (1H, br s, H-1″); δ_C 101.7, 72.3, 72.7, 74.0, 69.4, and 18.6 (C-1″-6″)], and a terminal β-D-glucopyranosyl unit [Glc (II): δ_H 5.12 (1H, d, J = 7.8 Hz, H-1‴); δ_C 105.0, 74.9, 78.1, 71.2, 78.4, and 62.0 (C-1‴-6‴)]. In the heteronuclear multiple bond correlation (HMBC) spectrum of **1**, long-range correlations were observed between H-1″ of Rha (δ_H 6.20) and C-2′ of Glc (I) (δ_C 77.3), H-1‴ of Glc (II) (δ_H 5.12) and C-4′ of Glc (I) (δ_C 81.9), and between H-1′ of Glc (I) (δ_H 4.91) and C-3 of the aglycone (δ_C 78.2) in **1**. Thus, **1** was deduced as (25*S*)-17α,27-dihydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.

Compound **2** ($C_{51}H_{82}O_{24}$) was obtained as an amorphous powder, and its ^{1}H- and ^{13}C-NMR spectra were indicative of the aglycone moiety of **2** being the same as that of **1**. However, the molecular formula of **2** was larger than that of **1** by $C_6H_{10}O_5$, which corresponded to one hexosyl unit. Analysis of the ^{1}H-^{1}H COSY, total correlation spectroscopy (TOCSY), HSQC, and HSQC-TOCSY spectra of the sugar moiety of **2** revealed that it was the configuration of a 2,4-disubstituted β-D-glucopyranosyl unit [Glc (I)], 3-monosubstituted α-L-rhamnopyranosyl unit (Rha), and two terminal β-D-glucopyranosyl units [Glc (II) and Glc (III)]. The HMBC spectrum of **2** exhibited long-range correlations between H-1⁗ of Glc (III) (δ_H 5.52) and C-3″ of Rha (δ_C 84.0), H-1″ of Rha (δ_H 6.20) and C-2′ of Glc (I) (δ_C 77.3), H-1‴ of Glc (II) (δ_H 5.13) and C-4′ of Glc (I) (δ_C 82.0), and between H-1′ of Glc (I) (δ_H 4.90) and C-3 of the aglycone (δ_C 78.3). Therefore, **2** was identified as (25*S*)-17α,27-dihydroxyspirost-5-en-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.

The ^{1}H- and ^{13}C-NMR spectral data of **3** ($C_{45}H_{70}O_{18}$) implied that **3** was analogous to **11**, including the diglycoside moiety attached to C-3 of the aglycone. However, the ^{13}C-NMR spectrum suggested that a substituent with six carbon atoms corresponding to the 3-hydroxy-3-methylglutaroyl (HMG) group was present in **3**; the signals arising from the HMG group included a methyl carbon at δ_C 28.3 (C-6‴), two methylene carbons at δ_C 46.4 (C-2‴) and 46.5 (C-4‴), a quaternary carbon with a hydroxy group at δ_C 70.0 (C-3‴), the carbonyl carbon of a carboxy group at δ_C 174.6 (C-5‴), and an ester carbonyl carbon at δ_C 171.6 (C-1‴). The HMG group was ascertained to be linked to C-27 of the aglycone based on a $^{3}J_{C,H}$ correlation between H_2-27 of the aglycone at δ_H 4.07 and 4.00 and C-1‴ of the HMG group at δ_C 171.6. Thus, **3** was identified as (25*R*)-17α-hydroxy-27-[(3-hydroxy-3-methylglutaroyl)oxy]-spirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Compound **4** ($C_{51}H_{80}O_{22}$) was shown to be a steroidal glycoside with an HMG group at the C-27 hydroxy group of the aglycone based on its ^{1}H- and ^{13}C-NMR spectra, as in the case of **3**. Its molecular formula was the same as that of **18**, and the spectroscopic features of **4** were very similar to those of **18**, except for the signals attributable to the

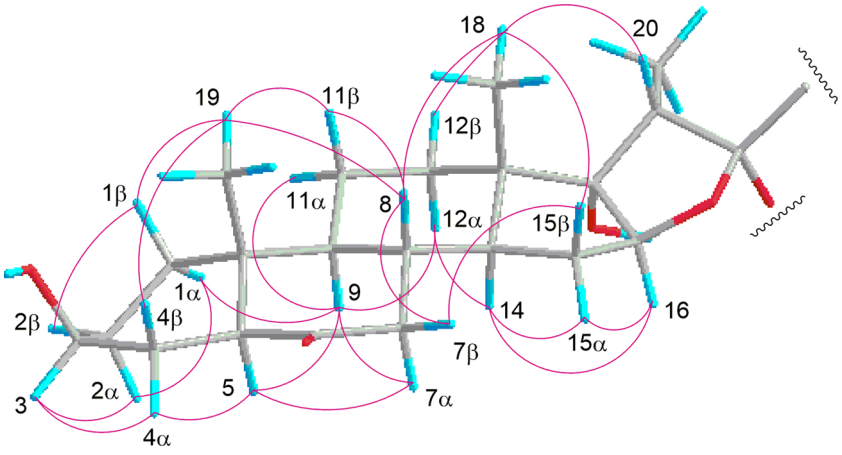

Fig. 2. Key NOE correlations of the A-, B-, C-, D-, and E-ring parts of **8a**.

triglycoside moiety attached to C-3 of the aglycone. The ^{1}H-^{1}H COSY and HSQC spectra indicated that **4** contained a 2,6-disubstituted inner β-D-glucopyranosyl unit [Glc (I): δ_H 4.97 (1H, d, J = 7.3 Hz, H-1′); δ_C 100.7, 77.5, 79.6, 71.6, 76.9, and 70.0 (C-1′-6′)], a terminal α-L-rhamnopyranosyl unit [Rha: δ_H 6.34 (1H, br s, H-1″); δ_C 102.0, 72.5, 72.8, 74.2, 69.5, and 18.7 (C-1″-6″)], and a terminal β-D-glucopyranosyl unit [Glc (II): δ_H 5.10 (1H, d, J = 7.8 Hz, H-1‴); δ_C 105.4, 75.2, 78.4, 71.7, 78.5, and 62.7 (C-1‴-6‴)]. In the HMBC spectrum of **4**, long-range correlations were observed between H-1‴ of Glc (II) (δ_H 5.10) and C-6′ of Glc (I) (δ_C 70.0), H-1″ of Rha (δ_H 6.34) and C-2′ of Glc (I) (δ_C 77.5), and between H-1′ of Glc (I) (δ_H 4.97) and C-3 of the aglycone (δ_C 78.4). Therefore, **4** was elucidated as (25*R*)-27-[(3-hydroxy-3-methylglutaroyl)oxy]-spirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside.

The ^{1}H- and ^{13}C-NMR spectral data of **5** ($C_{57}H_{90}O_{27}$) showed close similarity to those of **18**, including the signals attributed to the triglycoside moiety and HMG group, which were attached to C-3 and C-27 of the aglycone, respectively. However, the molecular formula of **5** was larger than that of **18** by $C_6H_{10}O_5$, and the presence of one more terminal β-D-glucopyranosyl unit [Glc (III): δ_H 5.28 (1H, d, J = 7.9 Hz, H-1⁗); δ_C 98.5, 75.3, 78.4, 71.6, 78.3, and 63.0 (C-1⁗-6⁗)] in **18** was verified based on ^{1}H- and ^{13}C-NMR spectral analysis. On comparison of the ^{13}C-NMR spectrum of **5** with that of **18**, it was found that the signal assigned to C-3⁗ of the HMG moiety was shifted downfield by 6.8 ppm, whereas those assigned to C-2⁗ and C-4⁗ had moved upfield by 2.4 and 2.1 ppm, respectively. Furthermore, a $^{3}J_{C,H}$ correlation was observed between H-1⁗ of Glc (III) (δ_H 5.28) and C-3⁗ of the HMG moiety (δ_C 76.7). These data implied that C-3⁗ of the HMG moiety was the position where the additional β-D-glucopyranosyl unit was linked. Thus, **5** was established as (25*R*)-[[3-(β-D-glucopyranosyl)oxy-3-methylglutaroyl]oxy]-spirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside. The absolute configuration of the HMG moiety in **3**-**5** have not yet been determined owing to their low yields. In the previous reports (Hong et al., 2012; Mimaki et al., 1993), the absolute configuration of the HMG moiety of related steroidal glycosides isolated from *L. brownii* var. *viridulum*, and *L. regale* and *L. henryi* was determined to be *S*. Therefore, the configuration of the HMG moiety in **3**-**5** was also speculated to be *S*.

Compound **6** ($C_{40}H_{64}O_{13}$) was obtained as an amorphous powder and had the same molecular formula as **13**. The ^{1}H- and ^{13}C-NMR spectral properties of **6** were very similar to those of **13**, except for the signals arising from the F-ring, suggesting that **6** was a stereoisomer of **13** at C-25. In the rotatory-frame Overhauser enhancement spectroscopy (ROESY) spectrum of **6**, ROE correlations were observed between H-23ax (δ_H 1.85) and H-20 (δ_H 1.96)/H-24eq (δ_H 1.46)/Me-27 (δ_H 1.09), Me-27 and H-24eq, and between H-24ax (δ_H 2.12) and H-23eq (δ_H 1.40)/H-25 (δ_H 1.93)/H-26 (δ_H 4.88). Therefore, **6** was formulated as (25*S*,26*R*)-26-methoxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

The chemical formula of **7** ($C_{39}H_{62}O_{13}$) was the same as that of **14**, the spectral features of **7** were closely related to those of **14**, suggesting that **7** was a stereoisomer of **14** with regard to the configuration at C-25. The nuclear Overhauser enhancement spectroscopy (NOESY) spectrum of **7** showed NOE correlations between H-20 (δ_H 1.92) and Me-18 (δ_H 0.81)/H-23ax (δ_H 1.51), and between H-23ax and H_2-27 (δ_H 4.16 and 3.97). Accordingly, **7** was elucidated as (25*R*)-27-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Compound **8** ($C_{45}H_{72}O_{19}$) was isolated as an amorphous powder. The ^{1}H-NMR spectrum of **8** exhibited signals for two angular methyl groups [δ_H 0.88 (3H, s, Me-18) and 0.78 (3H, s, Me-19)], two secondary methyl groups [δ_H 1.21 (3H, d, J = 7.2 Hz, Me-21) and 0.67 (3H, d, J = 5.4 Hz, Me-27)], and three anomeric protons [δ_H 6.18 (1H, br s), 5.10 (1H, d, J = 7.9 Hz), and 4.93 (1H, d, J = 7.3 Hz)]. The IR spectrum (1710 cm^{-1}) and ^{13}C-NMR spectrum (δ_C 209.4) indicated the presence of a carbonyl group in **8**. Enzymatic hydrolysis of **8** with naringinase yielded the aglycone (**8a**), and D-glucose and L-rhamnose as the carbohydrate moieties. The ^{1}H- and ^{13}C-NMR spectra of **8a** resembled those of (25*R*)-3β-hydroxy-5α-spirostan-6-one (laxogenin) (Baba et al., 2000), except for the signals arising from the D- and E-ring parts of the steroid skeleton. The molecular formula of **8a** was one oxygen atom more than that of laxogenin, and an oxygenated quaternary carbon (δ_C 89.8) was observed in the ^{13}C-NMR spectrum of **8a**. In the HMBC spectrum of **8a**, long-range correlations were observed from H-14 (δ_H 2.29), H-15α (δ_H 2.12), H-16 (δ_H 4.44), Me-18 (δ_H 0.93), H-20 (δ_H 2.27), Me-21 (δ_H 1.24) to δ_C 89.8, which was assigned to C-17. When the ^{13}C-NMR spectrum of **8a** was compared to that of laxogenin, C-13, C-16, and C-20 were shifted downfield, whereas C-14 and C-21 were shifted upfield. The above data indicated the presence of the C-17α hydroxy group. The C-3β and C-17α configurations, and A/B-*trans* ring junction of **8a** were ascertained by NOE correlations, as shown in Fig. 2. Thus, **8a** was established as (25*R*)-3β,17α-dihydroxy-5α-spirostan-6-one. As for the sugar moiety of **8**, the ^{1}H- and ^{13}C-NMR, and HMBC spectra indicated that the triglycoside linked to C-3 of the aglycone was the same as that of **1**. Compound **8** was determined as (25*R*)-17α-hydroxy-3-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl)oxy]-5α-spirostan-6-one.

The ^{1}H-NMR spectrum of **9** ($C_{45}H_{74}O_{18}$) exhibited signals for four methyl groups [δ_H 1.10 (3H, d, J = 6.9 Hz, Me-21), 1.09 (3H, d, J = 6.7 Hz, Me-27), 0.77 (3H, s, Me-19) and 0.56 (3H, s, Me-18)] and three anomeric protons [δ_H 6.20 (1H, d, J = 1.2 Hz), 5.12 (1H, d, J = 7.9 Hz), and 4.96 (1H, d, J = 7.2 Hz)]. The IR spectrum (1708 cm^{-1}) and ^{13}C-NMR spectrum (δ_C 213.9 and 209.5) indicated the presence of two carbonyl groups in **9**. Enzymatic hydrolysis of **9** with naringinase gave

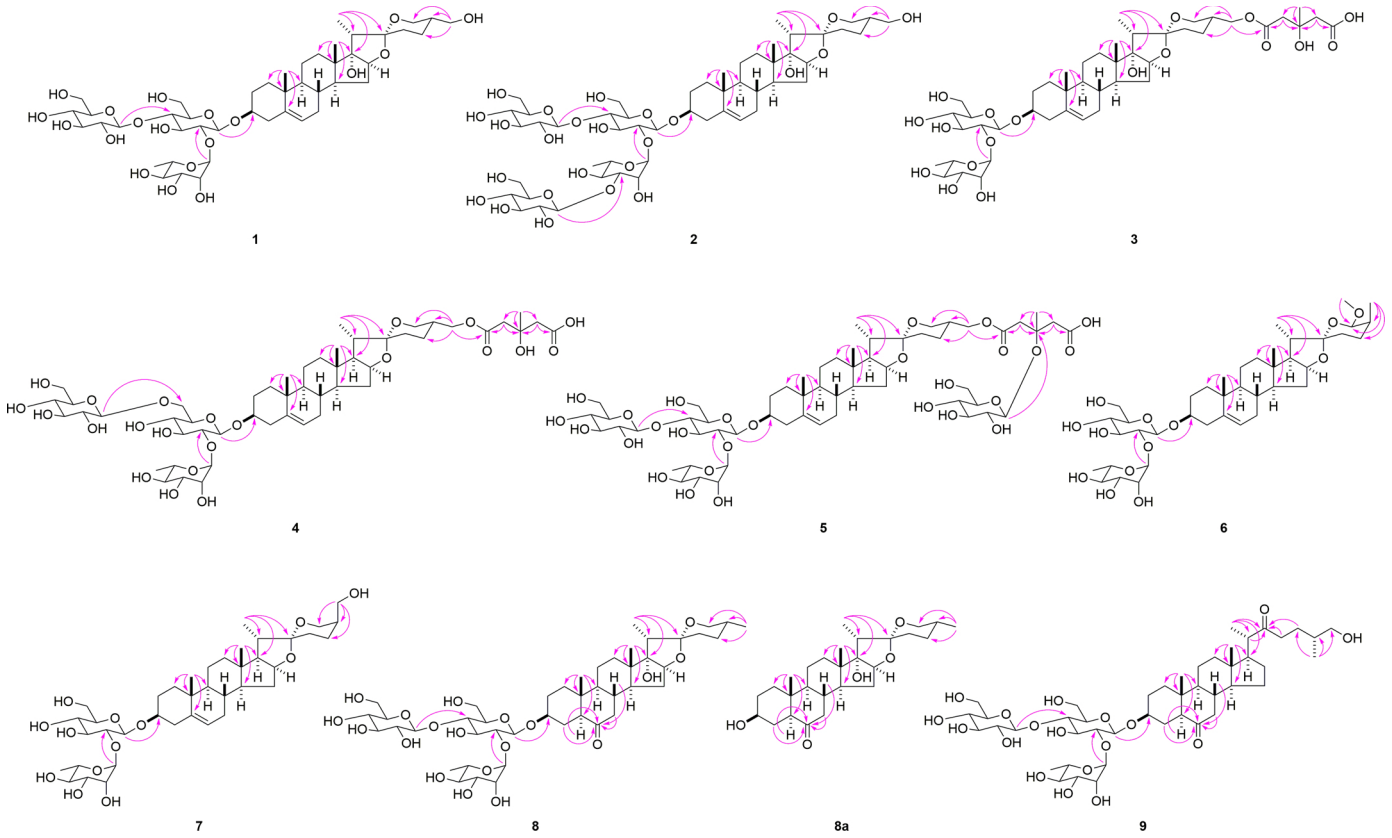

Fig. 3. Key HMBC correlations of **1**-**8**, **8a**, and **9**.

(25*R*)-3β,26-dihydroxy-5α-cholesta-6,22-dione (Shimomura et al., 1988) (**9a**), D-glucose and L-rhamnose. The sugar sequence attached to C-3 of the aglycone of **9** was determined to be identical to **1** by analysis of the ^{1}H- and ^{13}C-NMR, and HMBC spectra of **9** (Fig. 3). Thus, **9** was formulated as (25*R*)-26-hydroxy-3-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl)oxy]-5α-cholesta-6,22-dione.

The isolated compounds (**1**-**21**) and the aglycones (**1a**, **8a**, and **9a**) were evaluated for their cytotoxic activity against HL-60 and A549 cells, using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. It was found that **12** and **21** exhibited moderate cytotoxic activity toward HL-60 and A549 cells, with IC_{50} values of 6.9 ± 0.12 μM and 5.1 ± 0.031 μM against HL-60 cells, and 7.6 ± 0.14 μM and 4.4 ± 0.063 μM against A549 cells, respectively. Compound **20** only showed moderate cytotoxicity toward A549 cells, with an IC_{50} value of 6.7 ± 0.26 μM. Cisplatin was used as a positive control and gave IC_{50} values of 1.1 μM and 2.0 μM against HL-60 and A549 cells, respectively. The other compounds did not show any significant cytotoxic activity against HL-60 and A549 cells (IC_{50} > 10 μM).

3. Experimental

3.1. General

Optical rotations were obtained using a JASCO P-1030 (JASCO, Tokyo, Japan) automatic digital polarimeter. The IR spectra were recorded on a JASCO FT-IR 620 (JASCO). NMR spectra were recorded on a DRX-500 (500 MHz for ^{1}H-NMR) or a DPX-600 (600 MHz for ^{1}H-NMR) spectrometer (Bruker, Karlsruhe, Germany), using standard Bruker pulse programs at 300 K. Chemical shifts are reported in δ with reference to tetramethylsilane as an internal standard. HRESITOFMS data were obtained on a Waters-Micromass LCT mass spectrometer (Waters, Manchester, UK). CC was performed by Diaion HP-20 (50 mesh, Mitsubishi-Chemical, Tokyo, Japan), silica gel Chromatorex BW-300 (300 mesh, Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel COSMOSIL 75C_{18}-OPN (75 μM particle size, Nacalai Tesque, Kyoto, Japan). TLC were conducted on precoated silica gel 60 F_{254} or RP_{18} F_{254}S plates (0.25 mm thick, Merck, Darmstadt, Germany), and the spots were visualized by spraying the plates with 10 % H_2SO_4 aqueous solution, followed by heating. HPLC was performed with a system consisting of a CCPM pump (Shimadzu, Kyoto, Japan), an RI-8021 (Tosoh, Tokyo, Japan) or a Shodex OR-2 (Showa-Denko, Tokyo, Japan) detector, and a Rheodyne injection port (Rohnert Park, CA, USA). A TSK gel ODS-100Z column (10 mm i.d. × 250 mm, 5 μm; Tosoh) was used for preparative HPLC. The following materials and biochemical-grade reagents were used for the cell culture assays: Spectra Classic microplate reader (Tecan, Salzburg, Austria); 96-well microplate (Iwaki Glass, Chiba, Japan); HL-60 cells (JCRB 0085) and A549 cells (JCRB 0076) (Human Science Research Resources Bank, Osaka, Japan); fetal bovine serum (FBS), 0.25 % trypsin ethylenediaminetetraacetic acid (EDTA) solution, RPMI-1640 medium, minimum essential medium (MEM), cisplatin, and MTT (Sigma, St. Louis, MO, USA); penicillin G sodium salt and streptomycin sulfate (Gibco, Gland Island, NY, USA); paraformaldehyde and phosphate-buffered saline (PBS) (Wako Pure Chemical Industries, Osaka, Japan).

3.2. Plant material

L. speciosum bulbs were purchased from a garden center of Fujiengei (Okayama, Japan) in October 2014. A voucher specimen has been deposited at the herbarium of the Tokyo University of Pharmacy and Life Sciences (KS-2014-010).

3.3. Extraction and isolation

L. speciosum bulbs (fresh weight, 8.7 kg) were extracted with MeOH (9 L × 3) and the extracts were concentrated using an evaporator. The MeOH extract (340 g) was fractionated using the Diaion HP-20 column with MeOH/H_2O (3:7) (18 L), MeOH/H_2O (1:1) (12 L), MeOH (12 L), EtOH (6 L), and EtOAc (6 L), successively, as the eluent. The MeOH eluate fraction (45 g) was separated by ODS silica gel CC with MeCN/H_2O (2:3) to obtain four fractions; Fraction (Fr.) 1 (1.2 g), Fr. 2 (31.5 g), Fr. 3 (0.9 g), and Fr. 4 (10.9 g). Fr. 2 was subjected to silica gel CC eluted with $CHCl_3$/MeOH/H_2O (90:10:1, 40:10:1, 20:10:1) to give four subfractions (Frs. 2-1–4). Fraction 2-2 was purified by silica gel CC eluted with $CHCl_3$/MeOH/H_2O (40:10:1, 20:10:1), ODS silica gel CC eluted with MeCN/H_2O (3:7, 1:2, 2:3) and MeOH/H_2O (7:3), and preparative HPLC using MeCN/H_2O (3:7) or MeOH/H_2O (3:2, 7:3) to acquire **3** (3.3 mg), **7** (4.0 mg), **11** (12 mg), **14** (8.0 mg), and **17** (7.6 mg). Fraction 2-3 was separated by silica gel CC eluted with $CHCl_3$/MeOH/H_2O (40:10:1, 20:10:1), ODS silica gel CC eluted with MeCN/H_2O (1:2, 3:7) and MeOH/H_2O (7:3, 2:1, 3:2), and preparative HPLC using MeCN/H_2O (3:7) to collect **1** (61 mg), **8** (78 mg), **9** (14 mg), **15** (4.8 mg), **18** (168 mg), and **20** (2.1 mg). Fraction 2-4 was subjected to silica gel CC eluted with $CHCl_3$/MeOH/H_2O (30:10:1), ODS silica gel eluted with MeCN/H_2O (1:2, 3:7) and MeOH/H_2O (2:1, 3:2,), and preparative HPLC using MeCN/H_2O (3:7, 7:13) and MeOH/H_2O (2:1) to obtain **2** (6.7 mg), **4** (4.5 mg), **5** (6.9 mg), **16** (5.9 mg), and **21** (6.2 mg). Fr. 4 was chromatographed on silica gel eluted with $CHCl_3$/MeOH/H_2O (60:10:1, 30:10:1), ODS silica gel CC eluted with MeOH/H_2O (3:1, 4:1), and preparative HPLC using MeOH/H_2O (4:1, 3:1) to furnish **6** (3.9 mg), **10** (7.6 mg), **12** (7.3 mg), **13** (37 mg), and **19** (6.9 mg).

3.3.1. (25S)-17α,27-dihydroxyspirost-5-en-3β-yl O-α-L-rhamnopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (1)

Amorphous powder; $[\alpha]_D^{25}$ -70.5 (*c* 0.10, MeOH); IR (film) ν_{max}: 3390 (OH), 2934 (CH) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N): δ_H 5.27 (1H, br d, *J* = 4.1 Hz, H-6), 4.47 (1H, dd, *J* = 7.1, 6.7 Hz, H-16), 4.06 (1H, dd, *J* = 11.3, 4.7 Hz, H-26a), 3.88 (1H, dd, *J* = 11.3, 11.3 Hz, H-26b), 3.82 (1H, m, $W_{1/2}$ = 20.7 Hz, H-3), 3.72 (1H, dd, *J* = 10.6, 5.2 Hz, H-27a), 3.63 (1H, dd, *J* = 10.6, 7.4 Hz, H-27b), 2.29 (1H, q, *J* = 7.1 Hz, H-20), 1.23 (3H, d, *J* = 7.1 Hz, Me-21), 1.05 (3H, s, Me-19), 0.95 (3H, s, Me-18). For ^{1}H-NMR spectral data of the sugar moiety, see Table 1. For ^{13}C-NMR spectral data, see Table 2. HRESITOFMS *m/z*: 939.4557 $[M + Na]^+$ (calcd for $C_{45}H_{72}O_{19}Na$: 939.4566).

3.3.2. (25S)-17α,27-dihydroxyspirost-5-en-3β-yl O-β-D-glucopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (2)

Amorphous powder; $[\alpha]_D^{25}$ -52.2 (*c* 0.05, MeOH); IR (film) ν_{max}: 3389 (OH), 2928 (CH) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N): δ_H 5.43 (1H, br d, *J* = 4.9 Hz, H-6), 4.48 (1H, dd, *J* = 7.0, 6.7 Hz, H-16), 4.06 (1H, m, $W_{1/2}$ = 19.9 Hz, H-3), 4.06 (1H, dd, *J* = 10.9, 4.2 Hz, H-26a), 3.90 (1H, dd, *J* = 10.9, 10.9 Hz, H-26b), 3.74 (1H, dd, *J* = 10.7, 5.1 Hz, H-27a), 3.66 (1H, dd, *J* = 10.7, 7.4 Hz, H-27b), 2.30 (1H, q, *J* = 7.1 Hz, H-20), 1.25 (3H, d, *J* = 7.1 Hz, Me-21), 1.10 (3H, s, Me-19), 0.94 (3H, s, Me-18). For ^{1}H-NMR spectral data of the sugar moiety, see Table 1. For ^{13}C-NMR spectral data, see Table 2. HRESITOFMS *m/z*: 1101.5099 $[M + Na]^+$ (calcd for $C_{51}H_{82}O_{24}Na$: 1101.5094).

3.3.3. (25R)-17α-hydroxy-27-[(3-hydroxy-3-methylglutaroyl)oxy]-spirost-5-en-3β-yl O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (3)

Amorphous powder; $[\alpha]_D^{25}$ -42.9 (*c* 0.05, MeOH); IR (film) ν_{max}: 3405 (OH), 2931 (CH), 1725 and 1715 (C=O) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N): δ_H 5.30 (1H, br d, *J* = 4.9 Hz, H-6), 4.44 (1H, dd, *J* = 7.4, 6.4 Hz, H-16), 4.07 (1H, dd, *J* = 11.5, 5.3 Hz, H-27a), 4.00 (1H, dd, *J* = 11.5, 7.9 Hz, H-27b), 3.87 (1H, m, $W_{1/2}$ = 19.6 Hz, H-3), 3.83 (1H, dd, *J* = 11.0, 4.6 Hz, H-26a), 3.72 (1H, *J* = 11.0, 11.0 Hz, H-26b), 2.27 (1H, q, *J* = 7.2 Hz, H-20), 1.22 (3H, d, *J* = 7.2 Hz, Me-21), 1.09 (3H, s, Me-19), 0.95 (3H, s, Me-18). For ^{1}H-NMR spectral data of the sugar and HMG moieties, see Table 1. For ^{13}C-NMR spectral data, see Table 2. HRESITOFMS *m/z*: 921.4467 $[M + Na]^+$ (calcd for $C_{45}H_{70}O_{18}Na$: 921.4460).

3.3.4. (25R)-27-[(3-hydroxy-3-methylglutaroyl)oxy]-spirost-5-en-3β-yl O-α-L-rhamnopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (4)

Amorphous powder; $[\alpha]_D^{25}$ -29.0 (*c* 0.05, MeOH); IR (film) ν_{max}: 3355 (OH), 2926 (CH), 1730 and 1714 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N): δ_H 5.31 (1H, br d, *J* = 5.1 Hz, H-6), 4.48 (1H, m, H-16), 4.06 (1H, dd, *J* = 10.9, 3.1 Hz, H-27a), 4.02 (1H, dd, *J* = 10.9, 10.9 Hz, H-27b), 3.96 (1H, m, $W_{1/2}$ = 23.0 Hz, H-3), 3.90 (1H, dd, *J* = 11.2, 3.7 Hz, H-26a), 3.72 (1H, dd, *J* = 11.2, 11.2 Hz, H-26b), 1.12 (3H, d, *J* = 7.0 Hz, Me-21), 1.05 (3H, s, Me-19), 0.80 (3H, s, Me-18). For ^{1}H-NMR spectral data of the sugar and HMG moieties, see Table 1. For ^{13}C-NMR spectral data, see Table 2. HRESITOFMS *m/z*: 1067.5039 $[M + Na]^+$ (calcd for $C_{51}H_{80}O_{22}Na$: 1067.5039).

3.3.5. (25R)-[[3-(β-D-glucopyranosyl)oxy-3-methylglutaroyl]oxy]-spirost-5-en-3β-yl O-α-L-rhamnopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside (5)

Amorphous powder; $[\alpha]_D^{25}$ -17.2 (*c* 0.05, MeOH); IR (film) ν_{max}: 3388 (OH), 2926 (CH), 1727 and 1716 (C=O) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N): δ_H 5.28 (1H, br d, *J* = 4.7 Hz, H-6), 4.48 (1H, m, H-16), 4.03 (1H, m, H-27a), 3.95 (1H, br d, *J* = 11.3 Hz, H-27b), 3.87 (1H, m, $W_{1/2}$ = 22.1 Hz, H-3), 3.86 (1H, dd, *J* = 11.1, 3.7 Hz, H-26a), 3.68 (1H, dd, *J* = 11.1, 11.1 Hz, H-26b), 1.11 (3H, d, *J* = 6.9 Hz, Me-21), 1.05 (3H, s, Me-19), 0.81 (3H, s, Me-18). For ^{1}H-NMR spectral data of the sugar and HMG moieties, see Table 1. For ^{13}C-NMR spectral data, see Table 2. HRESITOFMS *m/z*: 1229.5587 $[M + Na]^+$ (calcd for $C_{57}H_{90}O_{27}Na$: 1229.5567).

3.3.6. (25S,26R)-26-methoxyspirost-5-en-3β-yl O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (6)

Amorphous powder; $[\alpha]_D^{25}$ -45.5 (*c* 0.05, MeOH); IR (film) ν_{max}: 3397 (OH), 2926 (CH) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N): δ_H 5.34 (1H, br d, *J* = 4.7 Hz, H-6), 4.88 (1H, d, *J* = 2.3 Hz, H-26), 4.68 (1H, m, H-16), 3.97 (1H, m, $W_{1/2}$ = 20.5 Hz, H-3), 3.50 (3H, s, OMe), 1.15 (3H, d, *J* = 7.0 Hz, Me-21), 1.09 (3H, d, *J* = 6.8 Hz, Me-27), 1.07 (3H, s, Me-19), 0.83 (3H, s, Me-18). For ^{1}H-NMR spectral data of the sugar moiety, see Table 1. For ^{13}C-NMR spectral data, see Table 2. HRESITOFMS *m/z*: 775.4252 $[M + Na]^+$ (calcd for $C_{40}H_{64}O_{13}Na$: 775.4245).

3.3.7. (25R)-27-hydroxyspirost-5-en-3β-yl O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (7)

Amorphous powder; $[\alpha]_D^{25}$ -58.3 (*c* 0.10, MeOH); IR (film) ν_{max}: 3433 (OH), 2935 (CH) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N): δ_H 5.31 (1H, br d, *J* = 4.8 Hz, H-6), 4.53 (1H, m, H-16), 4.16 (1H, br d, *J* = 10.5 Hz, H-27a), 4.14 (1H, dd, *J* = 11.2, 2.7 Hz, H-26a), 4.01 (1H, br d, *J* = 11.2 Hz, H-26b), 3.97 (1H, dd, *J* = 10.5, 6.8 Hz, H-27b), 3.94 (1H, m, $W_{1/2}$ = 20.2 Hz, H-3), 1.11 (3H, d, *J* = 7.0 Hz, Me-21), 1.05 (3H, s, Me-19), 0.81 (3H, s, Me-18). For ^{1}H-NMR spectral data of the sugar moiety, see Table 1. For ^{13}C-NMR spectral data, see Table 2. HRESITOFMS *m/z*: 761.4093 $[M + Na]^+$ (calcd for $C_{39}H_{62}O_{13}Na$: 761.4088).

3.3.8. (25R)-17α-hydroxy-3-[(O-α-L-rhamnopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl)oxy]-5α-spirostan-6-one (8)

Amorphous powder; $[\alpha]_D^{25}$ -57.5 (*c* 0.05, MeOH); IR (film) ν_{max}: 3388 (OH), 2925 (CH), 1710 (C=O) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N): δ_H 4.41 (1H, dd, *J* = 7.3, 7.3 Hz, H-16), 3.90 (1H, m, $W_{1/2}$ = 21.6 Hz, H-3), 3.49 (1H, dd, *J* = 10.4, 3.8 Hz, H-26a), 3.46 (1H, dd, *J* = 10.4, 10.4 Hz, H-26b), 2.23 (1H, q, *J* = 7.2 Hz, H-20), 1.21 (3H, d, *J* = 7.2 Hz, Me-21), 0.88 (3H, s, Me-18), 0.78 (3H, s, Me-19), 0.67 (3H, d, *J* = 5.4 Hz, Me-27). For ^{1}H-NMR spectral data of the sugar moiety, see Table 1. For ^{13}C-NMR spectral data, see Table 2. HRESITOFMS *m/z*:

Table 1
^{1}H-NMR spectral data for the sugar and HMG moieties of **1**-**9** in C_5D_5N.

Positions	1 δ_H	*J* (Hz)	Positions	2 δ_H	*J* (Hz)	Positions	3 δ_H	*J* (Hz)	Positions	4 δ_H	*J* (Hz)	Positions	5 δ_H	*J* (Hz)
Glc (I) 1′	4.91 d	7.6	Glc (I) 1′	4.90 d	7.3	Glc 1′	5.04 d	7.2	Glc (I) 1′	4.97 d	7.3	Glc (I) 1′	4.96 d	7.7
2′	4.17 dd	8.3, 7.6	2′	4.17 m		2′	4.28 dd	9.1, 7.2	2′	4.21 dd	8.8, 7.3	2′	4.18 dd	9.0, 7.7
3′	4.20 dd	8.3, 8.3	3′	4.18 m		3′	4.31 dd	9.1, 9.1	3′	4.24 dd	8.8, 8.8	3′	4.19 dd	9.0, 9.0
4′	4.21 dd	8.3, 8.3	4′	4.21 dd	8.8, 8.8	4′	4.19 dd	9.1, 9.1	4′	4.22 dd	8.8, 8.8	4′	4.21 dd	9.0, 9.0
5′	3.83 m		5′	3.77 m		5′	3.90 m		5′	4.00 m		5′	3.86 m	
6′a	4.51 dd	12.1, 3.6	6′a	4.52 br d	11.8	6′a	4.51 dd	12.1, 2.1	6′a	4.75 br d	10.9	6′a	4.53 br d	12.8
b	4.42 br d	12.1	b	4.45 br d	11.8	b	4.37 dd	12.1, 5.7	b	4.33 br d	10.9	b	4.47 br d	12.8
Rha 1″	6.20 br s		Rha 1″	6.20 br s		Rha 1″	6.39 br s		Rha 1″	6.34 br s		Rha 1″	6.26 br s	
2″	4.73 br d	3.0	2″	4.96 br d	3.3	2″	4.82 br s		2″	4.79 br s		2″	4.75 br s	
3″	4.57 dd	9.2, 3.0	3″	4.74 dd	9.5, 3.3	3″	4.64 dd	9.3, 3.1	3″	4.62 dd	9.3, 3.4	3″	4.60 dd	9.2, 3.5
4″	4.33 dd	9.2, 9.2	4″	4.54 dd	9.5, 9.5	4″	4.37 dd	9.3, 9.3	4″	4.35 dd	9.3, 9.3	4″	4.35 dd	9.2, 9.2
5″	4.91 m		5″	4.96 m		5″	5.00 m		5″	4.99 m		5″	4.95 m	
6″	1.74 d	6.1	6″	1.70 d	6.3	6″	1.78 d	5.9	6″	1.78 d	6.3	6″	1.77 d	6.2
Glc (II)1‴	5.12 d	7.8	Glc (II) 1‴	5.13 d	8.1	HMG 1‴	-		Glc (II) 1‴	5.10 d	7.8	Glc (II) 1‴	5.14 d	7.7
2‴	4.04 dd	8.3, 7.8	2‴	4.06 dd	8.8, 8.1	2‴a	3.15 d	14.3	2‴	4.04 dd	8.4, 7.8	2‴	4.07 dd	8.6, 7.7
3‴	4.25 dd	8.3, 8.3	3‴	4.22 dd	8.8, 8.8	b	3.12 d	14.3	3‴	4.23 dd	8.4, 8.4	3‴	4.29 dd	8.6, 8.6
4‴	4.26 dd	8.3, 8.3	4‴	4.29 dd	8.8, 8.8	3‴	-		4‴	4.14 dd	8.4, 8.4	4‴	4.23 dd	8.6, 8.6
5‴	3.96 m		5‴	3.95 m		4‴a	3.20 d	14.2	5‴	3.93 m		5‴	3.98 m	
6‴a	4.44 dd	13.4, 3.1	6‴a	4.46 br d	12.7	a	3.18 d	14.2	6‴a	4.52 br d	11.8	6‴a	4.48 br d	11.6
b	4.31 dd	13.4, 4.9	b	4.35 br d	12.7	5‴	-		b	4.33 br d	11.8	b	4.35 br d	11.6
						6‴	1.78 s							
			Glc (III) 1⁗	5.52 d	7.9				HMG 1⁗	-		HMG 1⁗	-	
			2⁗	4.11 dd	8.9, 7.9				2⁗a	3.16 d	14.3	2⁗a	3.30 d	15.2
			3⁗	4.21 dd	8.9, 8.9				b	3.12 d	14.3	b	3.20 d	15.2
			4⁗	4.33 dd	8.9, 8.9				3⁗	-		3⁗	-	
			5⁗	3.90 m					4⁗a	3.21 d	15.3	4⁗a	3.47 d	15.4
			6⁗a	4.42 br d	11.8				b	3.17 d	15.3	b	3.37 d	15.4
			b	4.39 br d	11.8				5⁗	-		5⁗	-	
									6⁗	1.78 s		6⁗	1.89 s	
												Glc (III) 1⁗	5.28 d	7.9
												2⁗	4.02 dd	8.5, 7.9
												3⁗	4.19 dd	8.5, 8.5
												4⁗	4.28 dd	8.5, 8.5
												5⁗	3.97 m	
												6⁗a	4.52 dd	11.9, 2.0
												b	4.32 dd	11.9, 4.4

Positions	6 δ_H	*J* (Hz)	Positions	7 δ_H	*J* (Hz)	Positions	8 δ_H	*J* (Hz)	Positions	9 δ_H	*J* (Hz)
Glc 1′	5.04 d	7.5	Glc 1′	5.06 d	7.2	Glc 1′	4.93 d	7.3	Glc 1′	4.96 d	7.2
2′	4.27 dd	9.0, 7.5	2′	4.29 dd	9.1, 7.2	2′	4.18 m		2′	4.20 dd	8.3, 7.2
3′	4.31 dd	9.0, 9.0	3′	4.31 dd	9.1, 9.1	3′	4.17 m		3′	4.22 dd	8.9, 8.3
4′	4.19 dd	9.0, 9.0	4′	4.19 dd	9.1, 9.1	4′	4.16 dd	8.8, 8.8	4′	4.27 dd	8.9, 8.9
5′	3.90 m		5′	3.91 m		5′	3.83 m		5′	3.86 m	
6′a	4.51 dd	11.7, 2.2	6′a	4.53 dd	11.8, 2.1	6′a	4.51 br d	12.4	6′a	4.52 br d	11.9
b	4.37 dd	11.7, 4.4	b	4.37 dd	11.8, 4.5	b	4.48 br d	12.4	b	4.33 br d	11.9
Rha 1″	6.38 br s		Rha 1″	6.40 br s		Rha 1″	6.18 br s		Rha 1″	6.20 d	1.2
2″	4.82 dd	3.3, 1.4	2″	4.82 br d	3.1	2″	4.69 dd	3.4, 1.4	2″	4.72 dd	3.4, 1.2
3″	4.64 dd	9.2, 3.3	3″	4.65 dd	9.3, 3.1	3″	4.58 dd	9.3, 3.4	3″	4.60 dd	9.4, 3.4
4″	4.36 dd	9.2, 9.2	4″	4.37 dd	9.3, 9.3	4″	4.30 dd	9.3, 9.3	4″	4.32 dd	9.4, 9.4
5″	5.00 m		5″	5.01 m		5″	4.88 m		5″	4.92 m	
6″	1.78 d	6.2	6″	1.79 d	6.2	6″	1.74 d	6.2	6″	1.77 d	6.2
						Glc 1‴	5.10 d	7.9	Glc 1‴	5.12 d	7.9
						2‴	4.03 dd	8.5, 7.9	2‴	4.06 dd	8.6, 7.9
						3‴	4.21 dd	9.0, 8.5	3‴	4.23 dd	8.6, 8.6
						4‴	4.25 dd	9.0, 9.0	4‴	4.26 dd	8.6, 8.6
						5‴	3.96 m		5‴	3.97 m	
						6‴a	4.44 dd	11.9, 2.3	6‴a	4.46 dd	11.9, 2.2
						b	4.31 dd	11.9, 4.9	b	4.31 dd	11.9, 5.5

500 MHz (**4** and **9**), 600 MHz (**1**-**3** and **5**-**8**).

939.4564 $[M + Na]^+$ (calcd for $C_{45}H_{72}O_{19}Na$: 939.4565).

3.3.9. *(25R)-3β,17α-dihydroxy-5α-spirostan-6-one (**8a**)*

Amorphous powder; $[\alpha]_D^{25}$ -45.1 (*c* 0.05, MeOH); IR (film) ν_{max}: 3291 (OH), 2928 (CH), 1702 (C=O) cm^{-1}; ^{1}H-NMR (600 MHz, C_5D_5N): δ_H 4.44 (1H, dd, *J* = 7.4, 6.2 Hz, H-16), 3.83 (1H, m, $W_{1/2}$ = 20.0 Hz, H-3), 3.51 (1H, dd, *J* = 10.6, 2.8 Hz, H-26a), 3.48 (1H, dd, *J* = 10.6, 10.6 Hz, H-26b), 2.44 (1H, dd, *J* = 12.2, 3.5 Hz, H-7β), 2.31 (1H, m, H-4α), 2.29 (1H, m, H-14), 2.27 (1H, q, *J* = 7.2 Hz, H-20), 2.25 (1H, br d, *J* = 11.8 Hz, H-5), 2.22 (1H, ddd, *J* = 12.9, 12.9, 3.7 Hz, H-

Table 2
^{13}C-NMR spectral data of **1**-**8**, **8a**, and **9**.

Positions	1	2	3	4	5	6	7	8	8a	9
1	37.5	37.5	37.6	37.5	37.5	37.5	37.5	36.7	37.0	36.7
2	30.1	30.2	30.2	30.0	30.1	30.2	30.2	29.2	31.8	29.3
3	78.2	78.3	78.2	78.4	78.1	77.9	78.0	76.4	70.0	76.4
4	38.8	39.1	39.0	39.1	38.9	38.9	39.0	26.5	31.2	26.4
5	140.7	140.8	141.0	140.9	140.8	140.8	141.0	56.3	56.9	56.3
6	121.7	122.0	121.7	121.6	121.8	121.7	121.7	209.4	210.1	209.5
7	32.4	32.4	32.3	32.1	32.3	32.3	32.4	46.8	46.9	46.6
8	32.3	32.3	31.8	31.1	31.6	31.6	31.8	37.9	38.1	37.6
9	50.2	50.2	50.2	50.1	50.3	50.2	50.4	53.5	53.6	53.7
10	37.1	37.1	37.2	37.1	37.1	37.1	37.2	40.9	40.9	40.9
11	20.9	21.0	20.9	21.0	21.1	21.0	21.1	21.3	21.4	21.5
12	32.0	32.1	32.1	39.8	39.8	39.8	39.8	31.8	32.0	39.5
13	45.1	45.1	45.2	40.4	40.4	40.4	40.5	45.7	45.8	43.1
14	52.9	53.0	53.0	56.5	56.6	56.6	56.7	52.9	52.9	56.0
15	31.7	31.8	32.4	32.2	32.1	32.1	32.2	31.2	31.3	24.3
16	90.0	90.1	90.1	81.2	81.2	81.2	81.5	89.6	89.6	27.6
17	90.1	90.2	90.1	62.8	62.8	62.7	62.7	89.8	89.8	52.3
18	17.1	17.2	17.1	16.3	16.3	16.3	16.3	17.2	17.2	12.1
19	19.4	19.5	19.4	19.4	19.4	19.4	19.4	13.1	13.2	13.0
20	44.8	44.9	44.8	41.9	41.9	42.4	42.2	44.7	44.7	49.4
21	9.7	9.7	9.6	14.9	14.9	14.9	14.9	9.7	9.7	16.6
22	110.2	110.2	109.9	109.4	109.4	109.8	111.9	109.8	109.8	213.9
23	31.7	31.8	31.4	30.3	31.1	27.2	25.6	32.0	31.9	39.8
24	23.5	23.6	23.2	21.4	23.6	21.6	26.4	28.7	28.7	27.7
25	38.9	39.0	35.3	35.5	35.4	36.2	31.7	30.3	30.4	36.1
26	63.9	63.9	62.9	63.0	63.0	60.6	99.5	66.6	66.6	67.3
27	64.3	64.4	66.0	66.1	66.2	61.4	10.5	17.1	17.2	17.2
OMe							55.8			
	Glc (I)	Glc (I)	Glc	Glc (I)	Glc (I)	Glc	Glc	Glc (I)		Glc (I)
1′	99.9	100.0	100.4	100.7	100.0	100.3	100.4	99.0		99.2
2′	77.3	77.3	77.9	77.5	77.3	77.8	77.9	77.6		77.5
3′	77.6	77.5	79.6	79.6	77.7	79.6	79.7	77.5		77.6
4′	81.9	82.0	71.9	71.6	82.1	71.8	71.9	82.0		82.0
5′	76.1	76.2	78.0	76.9	76.2	78.2	78.3	76.2		76.3
6′	61.8	62.1	62.7	70.0	61.9	62.6	62.7	61.9		62.0
	Rha	Rha	Rha	Rha	Rha	Rha	Rha	Rha		Rha
1″	101.7	101.7	102.0	102.0	101.8	102.0	102.1	101.9		101.9
2″	72.3	71.9	72.6	72.5	72.4	72.5	72.6	72.3		72.3
3″	72.7	84.0	72.9	72.8	72.8	72.8	72.9	72.7		72.7
4″	74.0	73.2	74.2	74.2	74.1	74.1	74.2	74.0		74.1
5″	69.4	69.3	69.5	69.5	69.5	69.5	69.5	69.4		69.5
6″	18.6	18.6	18.7	18.7	18.6	18.7	18.7	18.6		18.7
	Glc (II)	Glc (II)	HMG	Glc (II)	Glc (II)			Glc (II)		Glc (II)
1‴	105.0	107.0	171.6	105.4	105.2			105.1		105.2
2‴	74.9	75.0	46.4	75.2	75.0			74.9		74.9
3‴	78.1	78.4	70.0	78.4	78.5			78.2		78.3
4‴	71.2	71.2	46.5	71.7	71.2			71.2		71.2
5‴	78.4	78.4	174.6	78.5	78.4			78.4		78.5
6‴	62.0	61.9	28.3	62.7	62.1			62.0		62.1
		Glc (III)		HMG	HMG					
1⁗		105.2		171.6	171.4					
2⁗		76.0		46.4	43.9					
3⁗		78.5		70.0	76.7					
4⁗		71.3		46.5	44.3					
5⁗		78.4		174.6	173.8					
6⁗		62.3		28.3	25.2					
					Glc (III)					
1′′′′′					98.5					
2′′′′′					75.3					
3′′′′′					78.4					
4′′′′′					71.6					
5′′′′′					78.3					
6′′′′′					63.0					

125 MHz (**4** and **9**), 150 MHz (**1**-**3**, **5**-**8**, and **8a**).

12α), 2,12 (1H, m, H-15α), 2.05 (1H, br dd, J = 14.2, 12.4 Hz, H-2α), 2.03 (1H, m, H-7α), 2.01 (1H, m, H-8), 1.91 (1H, ddd, J = 12.5, 11.8, 11.8 Hz, H-4β), 1.73 (1H, ddd, J = 12.2, 12.2, 5.0 Hz, H-23a), 1.67 (1H, m, H-2β), 1.66 (1H, m, H-11α), 1.65 (1H, m, H-1β), 1.64 (1H, m, H-12β), 1.59 (1H, m, H-24a), 1.56 (1H, m, H-25), 1.52 (1H, m, H-24b), 1.51 (1H, m, H-23b), 1.45 (1H, ddd, J = 13.3, 13.3, 6.2 Hz, H-15β),

1.35 (1H, dddd, J = 12.9, 12.9, 12.9, 3.7 Hz, H-11β), 1.24 (3H, d, J = 7.2 Hz, Me-21), 1.21 (1H, br dd, J = 12.9, 12.9 Hz, H-9), 1.13 (1H, ddd, J = 14.2, 14.2, 4.1 Hz, H-1α), 0.93 (3H, s, Me-18), 0.79 (3H, s, Me-19), 0.67 (3H, d, J = 5.3 Hz, Me-27). For ^{13}C-NMR spectral data, see Table 2. HRESITOFMS *m/z*: 469.2931 [M + Na]$^+$ (calcd for $C_{27}H_{42}O_5Na$: 469.2930).

3.3.10. (25R)-26-hydroxy-3-[(O-α-L-rhamnopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranosyl)oxy]-5α-cholesta-6,22-dione (9)

Amorphous powder; $[\alpha]_D^{25}$ -49.5 (*c* 0.10, MeOH); IR (film) ν_{max}: 3327 (OH), 2926 (CH), 1708 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, C_5D_5N): δ_H 3.92 (1H, m, $W_{1/2}$ = 20.2 Hz, H-3), 3.77 (1H, dd, J = 10.5, 6.0 Hz, H-26a), 3.73 (1H, dd, J = 10.5, 6.0 Hz, H-26b), 1.10 (3H, d, J = 6.9 Hz, Me-21), 1.09 (3H, d, J = 6.7 Hz, Me-27), 0.77 (3H, s, Me-19), 0.56 (3H, s, Me-18). For ^{1}H-NMR spectral data of the sugar moiety, see Table 1. For ^{13}C-NMR spectral data, see Table 2. HRESITOFMS *m/z*: 925.4758 [M + Na]$^+$ (calcd for $C_{45}H_{74}O_{18}Na$: 925.4773).

3.3.11. Enzymatic hydrolysis of 1, 8, and 9

Compound **1** (8.0 mg) was treated with naringinase (160 mg, EC 232-962-4, Sigma) in AcOH/AcOK buffer (pH 4.3, 5.0 mL) at room temperature for 8 days. The reaction mixture was chromatographed on silica gel with $CHCl_3$/MeOH/H_2O (190:10:1) as the eluent to obtain **1a** (2.7 mg) and a sugar fraction (2.9 mg). The sugar fraction was analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan); solvent, MeCN/H_2O (85:15); detection, refractive index and optical rotation; flow rate, 0.8 mL/min. D-Glucose and L-rhamnose were identified by comparing their retention times (t_R) and optical rotations with those of the authentic samples: L-rhamnose (9.40, negative optical rotation) and D-glucose (18.6, positive optical rotation). Compounds **8** (25 mg) and **9** (7.0 mg) were independently subjected to enzymatic hydrolysis as described for **1** to obtain **8a** (5.2 mg) from **8**, and **9a** (2.4 mg) from **9**, and their sugar fractions. HPLC analyses of the sugar fractions under the same conditions as those used for **1** indicated the presence of D-glucose and L-rhamnose.

3.3.12. Acid hydrolysis of 2 and 6

Compounds **2** (1.0 mg) and **6** (1.0 mg) were independently dissolved in 1.0 M HCl (dioxane/H_2O, 1:1) and heated at 95 ℃ for 1 h under Ar atmosphere. Then, each reaction mixture was neutralized by passing through an Amberlite IRA-96 (Organo, Tokyo, Japan) column, and chromatographed on silica gel using $CHCl_3$/MeOH/H_2O (20:10:1 for **2**, 60:10:1 for **6**) as the eluent mixture to obtain the sugar fraction (0.46 mg from **2**, 0.32 mg from **6**). HPLC analyses of the sugar fractions were carried out under the same conditions as those used for **1**, except for the flow rate (1.0 mL/min). D-Glucose and L-rhamnose were identified by comparing their retention times (t_R) and optical rotations with those of the authentic samples: L-rhamnose (7.86, negative optical rotation) and D-glucose (15.9, positive optical rotation).

3.4. Cytotoxic activity assay

HL-60 and A549 cells were maintained in RPMI-1640 medium, and MEM, respectively. These cell media contained heat-inactivated 10 % (*v*/*v*) FBS supplemented with L-glutamine, penicillin G sodium salt (100 units/mL), and 100 μg/mL streptomycin sulfate. HL-60 and A549 cells were treated with each compound for 72 h, and cell viability was measured by MTT reduction assay, as previously described (Iguchi et al., 2017).

Declaration of Competing Interest

The authors declare no conflict of interest associated with this manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2020.01.008.

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