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Steroidal glycosides from the rhizomes of *Ruscus hypophyllum*

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Abstract

Seven steroidal glycosides, along with one known glycoside, were isolated from the rhizomes of *Ruscus hypophyllum* (Liliaceae). Comprehensive spectroscopic analysis, including 2D NMR spectroscopy, and the results of acid hydrolysis allowed the chemical structures of the compounds to be assigned as (23*S*,25*R*)-23-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**1**), 1β-hydroxyspirosta-5,25(27)-dien-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**2**), (22*S*)-16β,22-dihydroxycholest-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**3**), (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-22-hydroxycholest-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**4**), (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-22-hydroxycholest-5-en-3β-yl β-D-glucopyranoside (**5**), (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-3β,22-dihydroxycholest-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-(3,4-di-*O*-acetyl-β-D-xylopyranoside) (**6**), and (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-3β,22-dihydroxycholest-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-xylopyranoside (**7**), respectively. This is the first isolation of a series of cholestane glycosides from a *Ruscus* species.

Keywords: *Ruscus hypophyllum*; Liliaceae; Glycosides; Spirostanol saponins; Cholestane glycosides

1. Introduction

The genus *Ruscus* contains six species of flowering plants and belongs to the subfamily Asparagoideae in the Liliaceae. An alcohol extract of the rhizomes of *Ruscus aculeatus* has been used for the treatment of some veinous ailments for decades, and a variety of steroidal saponins based upon spirost-5-ene-1β,3β-diol have been isolated from the plant (Mimaki et al., 1998a,b,c,d, 1999c). Among the saponins from *R. aculeatus*, the bisdesmosidic spirostanol saponin aculeoside A is very unique in structure having 6-deoxy-D-glycero-L-threo-4-hexosulose as a carbohydrate component (Horikawa et al., 1994). *Ruscus hypophyllum* L. is distributed from the Madeira Islands to the Caucasian mountains and is cultivated for ornamental purposes. Although a few steroidal saponins were isolated from *R. hypophyllum* (Pkheidze et al., 1971), studies of this plant to date have been fragmentary and there has been no systematic exploration of its secondary metabolites. As part of our continuing investigation of plants of the genus *Ruscus*, we have now conducted a phytochemical screening of the rhizomes of *R. hypophyllum* and isolated two new spirostanol saponins and five new cholestane glycosides, together with one known glycoside. In this paper, we wish to report the structural determination of these compounds on the basis of extensive spectroscopic analysis, including 2D NMR data, and the results of acid hydrolysis.

2. Results and discussion

2.1. Isolation and structural elucidation of 1–8

Air-dried rhizomes of *R. hypophyllum* (1.2 kg) were extracted with MeOH under reflux. The concentrated MeOH extract (221 g) was subjected to porous-polymer polystyrene resin (Diaion HP-20) chromatography, and

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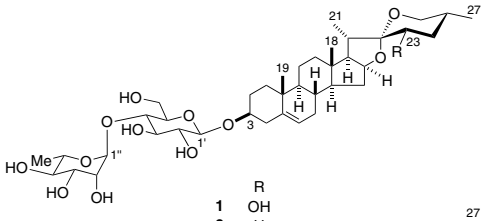
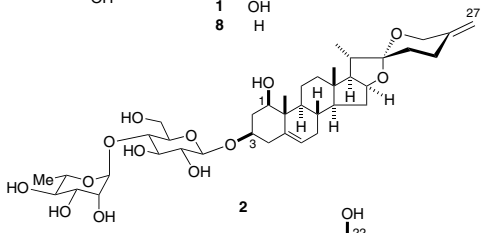
successively eluted with 30% MeOH in H_2O, EtOH, and EtOAc. The EtOH eluate (25 g), in which glycosides were enriched, was subjected to column chromatography on silica gel and ODS silica gel, as well as preparative HPLC, giving compounds **1** (6.0 mg), **2** (28.4 mg), **3** (79.2 mg), **4** (169 mg), **5** (8.5 mg), **6** (13.5 mg), **7** (12.1 mg), and **8** (15.1 mg). Compound **8** was identified as (25*R*)-spirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (Inoue et al., 1995a).



	R
1	OH
8	H



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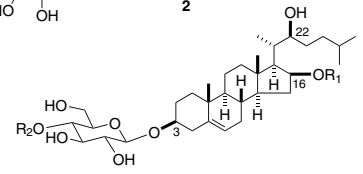
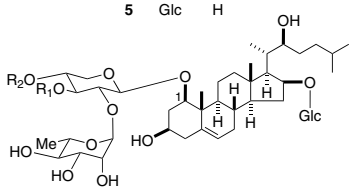
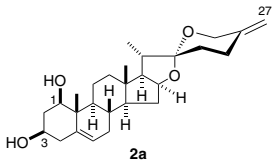


	R_1	R_2
3	H	Rha
4	Glc	Rha
5	Glc	H

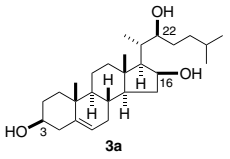


	R_1	R_2
6	Ac	Ac
7	Xyl	H

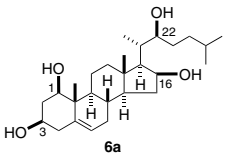
Rha: α-L-rhamnopyranosyl
Glc: β-D-glucopyranosyl
Xyl: β-D-xylopyranosyl



2a



3a



6a

Compound **1** was obtained as an amorphous solid. Its molecular formula was determined to be $C_{39}H_{62}O_{13}$ by HRESI–TOFMS (*m/z* 739.4230 $[M+H]^+$). The glycosidic nature of **1** was suggested by absorption bands at 3375 and 1060 cm^{-1} in the IR spectrum. The ^1H NMR spectrum of **1** showed two three-proton singlets at δ 0.99 and 0.83, indicating the presence of two angular methyl groups, as well as two three-proton doublets at δ 1.17 ($J = 7.0$ Hz) and 0.69 ($J = 6.1$ Hz), assignable to two secondary methyl groups. Furthermore, signals for an olefinic proton at δ 5.28 (*br d*, $J = 4.9$ Hz), two anomeric protons at δ 5.87 (*d*, $J = 1.0$ Hz) and 4.94 (*d*, $J = 7.8$ Hz), and the methyl group of a 6-deoxyhexopyranosyl moiety at δ 1.68 (*d*, $J = 6.2$ Hz) were observed. The ^1H NMR spectroscopic data and an acetalic carbon signal at δ 111.6 in the ^{13}C NMR spectrum of **1** are suggestive of a spirostanol diglycoside (Agrawal et al., 1985). Acid hydrolysis of **1** with 1.0 M HCl gave D-glucose and L-rhamnose, whereas the labile aglycone decomposed under acidic conditions and could not be obtained. Identification of the monosaccharides was carried out by direct HPLC analysis of the sugar fraction using an optical rotation (OR) detector. The ^1H and ^{13}C NMR spectroscopic data were closely related to those of the known compound **8**; however, slight differences were recognized in the signals due to the ring E and F parts between the two compounds. Furthermore, the molecular formula of **1** was higher than that of **8** by one oxygen atom and oxymethine signals were observed at δ_H 3.85 and δ_C 67.3. These data indicate the presence of a hydroxy group at the ring F part. The locus and configuration of the hydroxy group were established by analysis of the following spectroscopic data. In the ^1H–^1H COSY spectrum of **1**, the broad multiplet signal centered at δ 1.78, which was assignable to H-25, was shown to be coupled with the methylene protons at δ 2.07 (H-24eq) and 1.74 (H-24ax), as well as the oxymethylene protons at δ 3.52 (H-26eq) and 3.45 (H-26ax), and the secondary methyl group at δ 0.69 (Me-27) (see Fig. 1). The hydroxymethine proton at δ 3.85 showed proton spin-coupling correlations with the H_2-24 methylene protons. Thus, the presence of a hydroxy group at C-23 was verified. The large spin-coupling constants between H-23 and H-24ax (11.1 Hz), and between H-25 and H-26ax (10.9 Hz), and NOE correlations between H-14 and H-16/H-17, H-16 and H-17/H-26ax, H-17 and Me-21, Me-18 and H-20, H-23 and H-20/Me-

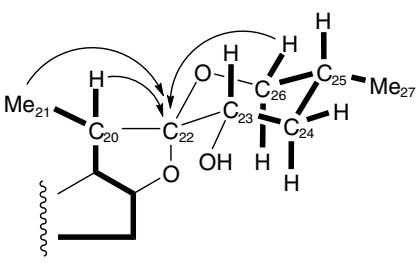


Fig. 1. Partial structure of **1**. Bold lines indicate the ^1H–^1H spin-couplings and arrows indicate $^1H/^{13}C$ long range correlations.

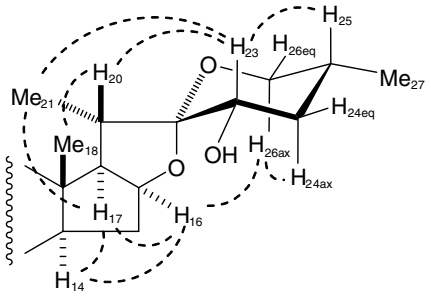


Fig. 2. Important NOE correlations of **1**.

21/H-25, and between H-24ax and H-26ax in the phase-sensitive NOESY spectrum of **1** gave evidence for the D/E *cis* ring junction, and the C-20α, C-22α, 23*S*, and 25*R* configurations (see Fig. 2). The sugar sequence of rhamnosyl-(1 → 4)-glucosyl and its linkage to C-3 of the aglycone were ascertained by long-range correlations between H-1 of the rhamnosyl unit at δ 5.87 and C-4 of the glucosyl unit at δ 78.1, and between H-1 of the glucosyl unit at δ 4.94 and C-3 of the aglycone at δ 78.2 in the HMBC spectrum of **1**. From the data presented above, the structure of **1** was determined to be (23*S*,25*R*)-23-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside.

Compound **2** was isolated as an amorphous solid with a molecular formula of $C_{39}H_{60}O_{13}$, as determined by HRESI–TOFMS (*m*/*z* 737.4139 $[M+H]^+$). The ^{1}H NMR spectrum contained signals for two tertiary methyl groups at δ 1.19 and 0.86 (*s*), a secondary methyl group at δ 1.02 (*d*, $J = 7.0$ Hz), exomethylene protons at δ 4.79 and 4.76 (each *br s*), and an olefinic proton at δ 5.54 (*br d*, $J = 5.7$ Hz), together with signals for two anomeric protons at δ 5.87 (*d*, $J = 1.0$ Hz) and 4.99 (*d*, $J = 7.8$ Hz). Acid hydrolysis of **2** with 1 M HCl gave the genuine aglycone spirosta-5,25(27)-diene-1β,3β-diol (neoruscogenin) (Mimaki et al., 1998a), and D-glucose and L-rhamnose as the carbohydrate moieties. The multiplet proton signal due to H-3 of the aglycone at δ 3.99 ($W_{1/2} = 22.4$ Hz) was shown to have proton spin-couplings with the methylene protons at δ 2.81 and 2.16 (H_2-2), and δ 2.71 and 2.52 (H_2-4), and was associated with the one-bond coupled carbon at δ 74.9 (C-3) by analysis of the HMQC spectrum. In the latter, long-range correlations were observed between H-1 of the rhamnosyl unit at δ 5.87 and C-4 of the glucosyl unit at δ 78.0, and between H-1 of the glucosyl unit at δ 4.99 and C-3 of the aglycone at δ 74.9. Thus, the structure of **2** was formulated as 1β-hydroxyspirosta-5,25(27)-dien-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside.

Compound **3** was shown to have the molecular formula $C_{39}H_{66}O_{12}$ on the basis of HRESI-TOFMS (*m*/*z* 727.4645 $[M+H]^+$). The ^{1}H NMR spectrum of **3** showed signals for five typical steroid methyl groups at δ 1.25 (*d*, $J = 6.9$ Hz), 1.13 (*s*), 0.92 (*s*), and 0.90 (3H × 2, *d*, $J = 6.5$ Hz), an olefinic proton at δ 5.34 (*br d*, $J = 4.9$ Hz), and two anomeric protons at δ 5.88 (*d*, $J = 1.4$ Hz) and 4.96 (*d*, $J = 7.8$ Hz). On acid hydrolysis of **3** with 1 M HCl, it was hydrolyzed to give (22*S*)-cholest-5-ene-3β,16β,22-triol (**3a**) (Kawashima et al., 1991) as the aglycone, and D-glucose and L-rhamnose as the carbohydrate moieties. A linkage of the diglycoside of rhamnosyl-(1 → 4)-glucosyl to C-3 of the aglycone as for **1** and **2** was confirmed by HMBC correlations between H-1 of the rhamnosyl unit at δ 5.88 and C-4 of the glucosyl unit at δ 78.1, and between H-1 of the glucosyl unit at δ 4.96 and C-3 of the aglycone at δ 78.2 (see Fig. 3). The structure of **3** was elucidated as (22*S*)-16β,22-dihydroxycholest-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside.

Compound **4** was analyzed as $C_{45}H_{76}O_{17}$ on the basis of HRESI–TOFMS (*m*/*z* 889.5142 $[M+H]^+$). The deduced molecular formula was higher than that of **3** by $C_6H_{10}O_5$, corresponding to one hexosyl unit. The ^{1}H NMR spectrum of **4** contained signals for three anomeric protons at δ 5.87 (*d*, $J = 0.6$ Hz), 4.94 (*d*, $J = 7.7$ Hz), and 4.73 (*d*, $J = 7.7$ Hz), as well as signals for five steroid methyl groups at δ 1.19 (*d*, $J = 7.0$ Hz), 0.97 (*s*), 0.91 (*d*, $J = 6.2$ Hz), 0.90 (*d*, $J = 6.2$ Hz), and 0.88 (*s*), and an olefinic proton at δ 5.24 (*br d*, $J = 4.7$ Hz). Acid hydrolysis of **4** with 1 M HCl gave **3a**, D-glucose, and L-rhamnose. On

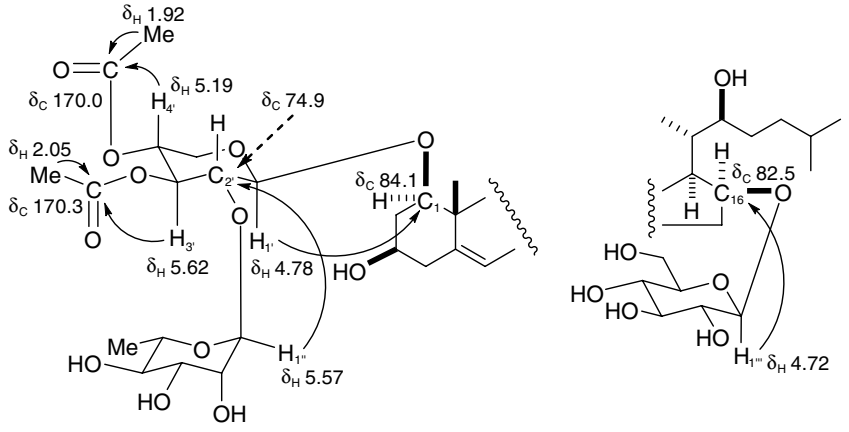


Fig. 3. HMBC correlations of the sugar moieties of **6**.

comparison of the ^{13}C NMR spectrum of **4** with that of **3**, a set of six signals corresponding a terminal β-D-rhamnopyranosyl unit could be observed at δ 106.8 (CH), 75.5 (CH), 78.6 (CH), 71.5 (CH), 78.2 (CH), and 62.7 (CH_2) in addition to the signals due to a rhamnosyl-(1 → 4)-glucosyl unit, of which was supposed to be linked to C-3 of the aglycone. In the ^1H NMR spectrum of **4**, the resonance at δ 4.53 (*ddd*, $J = 7.7$, 7.7, 4.5 Hz) was attributable to H-16, which had an HMBC correlation with C-13 at δ 42.3 and was associated with the one-bond coupled carbon resonation at δ 82.5 by the HMQC spectrum. In the HMBC spectrum of **4**, H-1 of the additional glucosyl unit at δ 4.73 showed a long-range correlation with C-16 of the aglycone at δ 82.5. Furthermore, long-range correlations were observed between H-1 of the rhamnosyl unit at δ 5.87 and C-4 of the glucosyl unit at δ 78.1, and between H-1 of the glucosyl unit at δ 4.94 and C-3 of the aglycone at δ 78.2. Accordingly, the structure of **4** was shown to be (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-22-hydroxycholest-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside.

Compound **5** ($C_{39}H_{66}O_{13}$) was suggested to be a diglycoside of **3a** from its spectroscopic data. When the ^{13}C NMR spectrum of **5** was compared with that of **4**, the signals due to the terminal rhamnosyl unit disappeared in **5**. Furthermore, the signal due to C-4 of the glucosyl group attached to C-3 of the aglycone was shifted upfield by 6.6 ppm, whereas those due to C-3 and C-5 moved downfield by 1.8 and 1.3 ppm, respectively. All other signals were observed at almost the same positions. In the HMBC spectrum of **5**, H-1 of one glucosyl unit at δ 5.01 (*d*, $J = 7.7$ Hz) showed a long-range correlation with C-3 of the aglycone at δ 78.0 and that of another glucosyl unit at δ 4.72 (*d*, $J = 7.8$ Hz) had a correlation with C-16 of the aglycone at δ 82.4. Acid hydrolysis of **5** with 1 M HCl gave **3a** and D-glucose. Thus, the structure of **5** was assigned as (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-22-hydroxycholest-5-en-3β-yl β-D-glucopyranoside.

Compound **6** was deduced to be $C_{48}H_{78}O_{19}$ from HRESI–TOFMS (*m/z* 959.5245 $[M+H]^+$). The ^1H NMR spectrum of **6** showed signals for five steroid methyl groups at δ 1.34 (*s*), 1.17 (*d*, $J = 6.9$ Hz), 1.06 (*s*), and 0.89 (3H × 2, *d*, $J = 6.2$ Hz), and three anomeric protons at δ 5.57 (*d*, $J = 1.6$ Hz), 4.78 (*d*, $J = 7.2$ Hz), and 4.72 (*d*, $J = 7.6$ Hz). Two three-proton singlet signals at δ 2.05 and 1.92 were, respectively, correlated to the δ 20.7 and 20.5 resonances in the HMQC spectrum and to δ 170.3 and 170.0 in the HMBC spectrum, indicating that **6** has two acetyl groups. Acid hydrolysis of **6** with 1 M HCl gave (22*S*)-cholest-5-en-1β,3β,16β,22-tetrol (**6a**) (Kawashima et al., 1991) as the aglycone, and D-glucose, L-rhamnose, and D-xylose as the carbohydrate moieties. The results of acid hydrolysis and sequential ^1H NMR spectroscopic assignments from H-1 to H_2-5, H-1 to Me-6, and H-1 to H_2-6 of the monosaccharides, including their signal multiplet patterns and coupling constants, allowed the identification of a terminal α-L-rhamnopyranosyl unit, a terminal β-D-glucopyranosyl unit, and a substituted β-D-xylopyranosyl unit in **6**. In the HMBC spectrum of **6**, the signal at δ 3.66 (*dd*, $J = 12.1$, 3.7 Hz) showed long-range correlations with C-5 at δ 138.9, C-9 at δ 50.2, C-10 at δ 42.6, and C-19 at δ 14.8 and was assigned to H-1. The resonance at δ 4.53 (*m*) was assigned to H-16, which had a long-range correlation with C-13 at δ 42.0. The signals due to H-1 and H-16 were correlated to the respective carbon resonances at δ 84.1 (C-1) and 82.5 (C-16) in the HMQC spectrum, to which the sugars were linked. The H-1 signal due to the glucosyl moiety at δ 4.72 showed a long-range correlation with C-16, whereas that due to the rhamnosyl residue at δ 5.57 gave an HMBC correlation with the δ 74.9 resonance assignable to C-2 of the xylosyl group, whose H-1 proton at δ 4.78, in turn, was correlated to C-1 of the aglycone. The ^1H NMR signals assignable to H-3 and H-4 of the xylosyl residue appeared at the significantly lower positions of δ 5.62 and 5.19, respectively. Furthermore, HMBC correlations were observed between δ_H 5.62 and $\delta_{C=O}$ 170.3, and between δ_H 5.19 and $\delta_{C=O}$ 170.0. These data imply that the acetyl groups are involved in an ester linkage at C-3 and C-4 of the xylosyl group. Thus, the structure of **6** was defined as (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-3β,22-dihydroxycholest-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-(3,4-di-*O*-acetyl-β-D-xylopyranoside).

Compound **7** exhibited a molecular formula of $C_{49}H_{82}O_{21}$ based on HRESI–TOFMS (*m/z* 1007.5375 $[M+H]^+$). The ^1H and ^{13}C NMR spectroscopic properties of the aglycone moiety of **7** showed a close similarity to those of **6**. However, signals for four anomeric protons and carbons were observed at δ_H 6.40 (*d*, $J = 1.5$ Hz)/δ_C 101.5, δ_H 4.93 (*d*, $J = 7.7$ Hz)/δ_C 105.1, δ_H 4.72 (*d*, $J = 7.8$ Hz)/δ_C 106.9, and δ_H 4.65 (*d*, $J = 7.6$ Hz)/δ_C 100.7. Acid hydrolysis of **7** with 1 M HCl gave **6a**, D-glucose, L-rhamnose, and D-xylose. The results of acid hydrolysis and sequential ^1H NMR assignments from H-1 to H_2-5, H-1 to Me-6, and H-1 to H_2-6 of the monosaccharides, including their signal multiplet patterns and coupling constants, allowed the identification of a terminal α-L-rhamnopyranosyl unit, a terminal β-D-glucopyranosyl unit, a terminal β-D-xylopyranosyl unit, and a substituted β-D-xylpyranosyl unit in **7**. In the HMBC spectrum of **7**, the H-1 protons of the rhamnosyl group at δ 6.40 and xylosyl group at δ 4.93 showed long-range correlations with C-2 and C-3 of the substituted xylosyl unit at δ 75.6 and 88.5, respectively, whose H-1 proton, in turn, exhibited a correlation with C-1 of the aglycone at δ 84.1. On the other hand, a long-range correlation was observed between H-1 of the glucosyl unit at δ 4.72 and C-16 of the aglycone at δ 82.6. Accordingly, the structure of **7** was elucidated as (22*S*)-16β-[(β-D-glucopyranosyl)oxy]-3β,22-dihydroxycholest-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-xylopyranoside.

2.2. Conclusions

Compound **2** is a rare type of steroidal saponin based upon spirost-5-ene-1β,3β-diol (ruscogenin) with a sugar

unit at C-3 of the aglycone. Although a variety of steroidal saponins based upon ruscogenin have been isolated from plants of the genus *Ruscus*, glycosides of cholestane derivatives have not been reported, except for one cholestane bisdesmoside from *Ruscus hypoglossum* (Mimaki et al., 1999b). This is the first isolation of a series of cholestane glycosides from a *Ruscus* species.

Cholestane glycosides have been isolated from the several species of the genus *Allium* (Kawashima et al., 1991; Mimaki et al., 1993, 1999a; Inoue et al., 1995a,b), *Camassia* (Kuroda et al., 2001a), *Convallaria* (Higano et al., 2007), *Galtonia* (Kuroda et al., 2001b; Mimaki et al., 2001), *Ornithogalum* (Kubo et al., 1992a,b; Kuroda et al., 1999, 2002, 2004), and *Reinekea* (Kanmoto et al., 1994), which belong to the family Liliaceae, as well as those of *Dracaena* (Mimaki et al., 1997), *Nolina* (Takaashi et al., 1995), and *Polianthes* (Mimaki et al., 2000), which are classified to the family Agavaceae. On the basis of the facts reported up to the present, cholestane glycosides are considered to be distributed in the limited species belonging to the family Liliaceae and the taxonomically related family Agavaceae.

3. Experimental

3.1. General

Optical rotations were measured using a JASCO P-1030 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO FT-IR 620 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H NMR, Karlsruhe, Germany) using standard Bruker pulse programs. Chemical shifts are given as δ-value with reference to tetramethylsilane (TMS) as internal standard. HRESI–TOFMS data were obtained on a Micromass LCT mass spectrometer (Manchester, UK). Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai Tesque, Kyoto, Japan) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 followed by heating. HPLC was performed by using a system comprised of a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), an RI-8010 detector (Tosoh) or a Shodex OR-2 detector (Showa-Denko, Tokyo, Japan), and a Rheodyne injection port. A Capcell Pak C_{18} UG120 column (10 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan) was employed for preparative HPLC.

3.2. Plant material

The rhizomes of *R. hypophyllum* were collected in the fields of Giresun, Tirebolu, and Inkoy wards, Turkey, in March 2002. The plant was identified by one of the authors (M.C.). A voucher specimen of the plant has been deposited in Ankara Universitesi Eczacilik Fakultesi Herbaryumu (No. 22844).

3.3. Extraction and isolation

The powdered rhizomes (dry weight, 1.2 kg) were macerated in MeOH (4.5 l) at room temperature for 1 week. The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (221 g) was passed through a Diaion HP-20 column (70 mm i.d. × 380 mm), and successively eluted with MeOH H_2O (3:7, v/v), EtOH, and EtOAc. Column chromatography of the EtOH eluate (25 g) on silica gel (70 mm i.d. × 270 mm) and elution with a stepwise gradient mixture of $CHCl_3$–MeOH (5:1; 3:1; 1:1), and finally with MeOH alone, gave eight fractions (frs. I–VIII). Fr. II was subjected to column chromatography on silica gel (40 mm i.d. × 230 mm) eluted with $CHCl_3$–MeOH–H_2O (50:10:1; 30:10:1) and ODS silica gel (40 mm i.d. × 230 mm) with MeCN–H_2O (2:1; 5:8) to yield **1** (6.0 mg) and **8** (15.1 mg). Fr. III was subjected to silica gel column chromatography (25 mm i.d. × 230 mm) eluted with $CHCl_3$–MeOH–H_2O (50:10:1) and ODS silica gel column chromatography (40 mm i.d. × 190 mm) with MeCN–H_2O (1:1; 5:7; 5:8) and MeOH–H_2O (7:3) to give **2** (28.4 mg), **5** (8.5 mg), and **6** (13.5 mg). Fr. IV was applied to a silica gel (40 mm i.d. × 200 mm) column eluted with $CHCl_3$–MeOH–H_2O (30:10:1), as well as and ODS silica gel column chromatography (20 mm i.d. × 230 mm) with MeOH–H_2O (8:5; 4:3) and MeCN–H_2O (5:9; 5:11) as eluents to afford **3** (79.2 mg) and **4** (169 mg). Fraction VII was subjected to a silica gel column (40 mm i.d. × 230 mm) eluted with $CHCl_3$–MeOH–H_2O (30:10:1), an ODS silica gel column (10 mm i.d. × 250 mm) with MeCN–H_2O (1:1; 1:2; 1:4), and to preparative HPLC using MeCN–H_2O (10:27) to furnish **7** (12.1 mg).

3.4. Compound *1*

(23*S*,25*R*)-23-Hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**1**); colorless amorphous solid; $[\alpha]_D^{25}$ −78.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3375 (OH), 2927 and 2904 (CH), 1060; ^{1}H NMR (C_5D_5N): δ 5.28 (1H, *br d*, $J = 4.9$ Hz, H-6), 4.62 (1H, *q*-like, $J = 7.8$ Hz, H-16), 3.85 (1H, *dd*, $J = 11.1$, 4.8 Hz, H-23), 3.84 (1H, *br m*, $W_{1/2} = 19.8$ Hz, H-3), 3.52 (1H, *dd*, $J = 10.9$, 2.9 Hz, H-26eq), 3.45 (1H, *dd*, $J = 10.9$, 10.9 Hz, H-26ax), 3.01 (1H, *m*, H-20), 2.07 (1H, *m*, H-26eq), 1.86 (1H, *dd*, $J = 7.8$, 7.0 Hz, H-17), 1.78 (1H, *m*, H-25), 1.74 (1H, *m*, H-24ax), 1.17 (3H, *d*, $J = 7.0$ Hz, Me-21), 1.05 (1H, *m*, H-14), 0.99 (3H, *s*, Me-18), 0.83 (3H, *s*, Me-19), 0.69 (3H, *d*, $J = 6.1$ Hz, Me-27); For ^{1}H NMR spectroscopic data of the sugar moiety, see Table 1; for ^{13}C NMR (C_5D_5N) spectroscopic data, see Table 2; HRESI–TOFMS *m/z*: 739.4230 $[M+H]^+$ (calculated for $C_{39}H_{63}O_{13}$, 723.4269).

Table 1
^{1}H NMR spectroscopic data for the sugar moieties of **1** and **4**–**7** in C_5D_5N

1					4					5					6					7				
		δ		*J* (Hz)			**δ**		*J* (Hz)			**δ**		*J* (Hz)			**δ**		*J* (Hz)			**δ**		*J* (Hz)
Glc	1′	4.94	*d*	7.8	*Glc*	1′	4.94	*d*	7.7	*Glc*	1′	5.01	*d*	7.7	*Xyl*	1′	4.78	*d*	7.2	*Xyl*	1′	4.65	*d*	7.6
	2′	3.96	*dd*	9.2, 7.8		2′′	3.96	*dd*	9.2, 7.7		2′	4.02	*dd*	9.3, 7.7		2′	4.15	*dd*	9.0, 7.2		2′	4.11	*dd*	8.9, 7.6
	3′	4.20	*dd*	9.2, 9.2		3′	4.21	*dd*	9.2, 9.2		3′	4.31	*dd*	9.3, 9.3		3′	5.62	*dd*	9.0, 9.0		3′	3.94	*dd*	8.9, 8.9
	4′	4.41	*dd*	9.2, 9.2		4′	4.42	*dd*	9.2, 9.2		4′	4.25	*dd*	9.3, 9.3		4′	5.19	*ddd*	9.0, 8.0, 5.2		4′	3.90	*ddd*	9.8, 8.9, 4.7
	5′	3.69	*ddd*	9.2, 3.6, 1.6		5′	3.70	*ddd*	9.2, 3.4, 2.6		5′	3.94	*ddd*	9.3, 5.3, 2.2		5′a	4.24	*dd*	11.8, 5.2		5′a	4.24	*dd*	11.1, 4.7
	6′a	4.24	*dd*	12.6, 1.6		6′a	4.24	*dd*	12.6, 2.6		6′a	4.51	*dd*	11.9, 2.2		5′b	3.56	*dd*	11.8, 8.0		5′b	3.47	*dd*	11.1, 9.8
	6′b	4.14	*dd*	12.6, 3.6		6′b	4.14	*dd*	12.6, 3.4		6′b	4.39	*dd*	11.9, 5.3										
Rha	1′′	5.87	*d*	1.0	*Rha*	1′′	5.87	*d*	0.6	*Glc*	1′′	4.72	*d*	7.8	*Rha*	1′′	5.57	*d*	1.6	*Rha*	1′′	6.40	*d*	1.5
	2′′	4.71	*dd*	3.2, 1.0		2′′	4.70	*dd*	3.1, 0.6		2′′	3.98	*dd*	9.2, 7.8		2′′	4.49	*dd*	3.1, 1.6		2′′	4.79	*dd*	3.3, 1.5
	3′′	4.58	*dd*	9.4, 3.2		3′′	4.58	*dd*	9.4, 3.1		3′′	4.16	*dd*	9.2, 9.2		3′′	4.46	*dd*	9.3, 3.1		3′′	4.59	*dd*	9.4, 3.3
	4′′	4.34	*dd*	9.4, 9.4		4′′	4.34	*dd*	9.4, 9.4		4′′	4.25	*dd*	9.2, 9.2		4′′	4.28	*dd*	9.3, 9.3		4′′	4.30	*dd*	9.4, 9.4
	5′′	4.96	*dq*	9.4, 6.2		5′′	4.98	*dq*	9.4, 6.2		5′′	3.80	*ddd*	9.2, 5.1, 2.4		5′′	4.62	*dq*	9.3, 6.0		5′′	4.80	*dq*	9.4, 6.1
	6′′	1.68	*d*	6.2		6′′	1.69	*d*	6.2		6′′a	4.45	*dd*	11.7, 2.4		6′′	1.72	*d*	6.0		6′′	1.74	*d*	6.1
											6′′b	4.37	*dd*	11.7, 5.1										
					Glc	1′′′	4.73	*d*	7.7						*Glc*	1′′′	4.72	*d*	7.6	*Xyl*	1′′′	4.93	*d*	7.7
						2′′′	3.98	*dd*	9.3, 7.7							2′′′	3.98	*dd*	9.3, 7.6		2′′′	3.96	*dd*	8.5, 7.7
						3′′′	4.16	*dd*	9.3, 9.3							3′′′	4.17	*dd*	9.3, 9.3		3′′′	4.15	*dd*	8.5, 8.5
						4′′′	4.26	*dd*	9.3, 9.3							4′′′	4.25	*dd*	9.3, 9.3		4′′′	4.12	*ddd*	10.5, 8.5, 4.2
						5′′′	3.81	*ddd*	9.3, 4.8, 2.4							5′′′	3.79	*ddd*	9.3, 4.8, 2.4		5′′′a	4.22	*dd*	10.5, 4.2
						6′′′a	4.46	*dd*	11.7, 2.4							6′′′a	4.45	*dd*	11.6, 2.4		5′′′b	3.67	*dd*	10.5, 10.5
						6′′′b	4.39	*dd*	11.7, 4.8							6′′′b	4.38	*dd*	11.6, 4.8					
															Ac		2.05	*s*		*Glc*	1′′′′	4.72	*d*	7.8
																	1.92	*s*			2′′′′	3.98	*dd*	9.2, 7.8
																					3′′′′	4.16	*dd*	9.2, 9.2
																					4′′′′	4.26	*dd*	9.2, 9.2
																					5′′′′	3.80	*ddd*	9.2, 4.9, 2.4
																					6′′′′a	4.46	*dd*	11.7, 2.4
																					6′′′′b	4.38	*dd*	11.7, 4.9

Table 2
^{13}C NMR spectroscopic data for **1**–**7** in C_5D_5N

		1		**2**		**3**		**4**		**5**		**6**		**7**
1		37.3		77.6		37.4		37.3		37.3		84.1		84.1
2		30.0		40.8		30.1		30.1		30.1		37.0		37.3
3		78.2		74.9		78.2		78.2		78.0		67.9		68.2
4		39.1		39.6		39.2		39.2		39.2		43.5		43.7
5		140.7		139.0		140.9		140.7		140.7		138.9		139.2
6		121.6		125.0		121.8		121.9		121.8		125.0		124.9
7		32.0		32.1		32.1		32.0		31.9		31.6		31.6
8		31.5		32.7		31.8		31.6		31.6		33.1		33.1
9		50.1		51.0		50.4		50.3		50.3		50.2		50.2
10		36.9		43.5		36.9		36.9		36.8		42.6		42.6
11		21.0		24.0		21.0		21.0		21.0		24.0		24.0
12		40.0		40.3		40.3		39.9		39.9		40.5		40.5
13		40.9		40.1		42.9		42.3		42.3		42.0		42.1
14		56.5		56.7		54.7		55.0		55.0		55.1		55.2
15		32.1		32.2		37.6		36.8		36.8		37.1		37.1
16		81.5		81.3		71.3		82.5		82.4		82.5		82.6
17		62.3		63.0		59.6		57.8		57.8		57.9		58.0
18		16.4		16.4		13.3		13.4		13.4		13.7		13.8
19		19.2		13.6		19.3		19.3		19.3		14.8		15.0
20		35.6		41.7		37.4		35.8		35.8		35.8		35.9
21		14.6		14.9		13.4		12.5		12.4		12.5		12.5
22		111.6		109.3		73.2		73.0		73.0		73.0		73.1
23		67.3		33.1		29.9		33.7		33.7		33.6		33.6
24		38.6		28.8		36.4		36.6		36.5		36.6		36.6
25		31.4		144.3		28.4		28.8		28.8		28.8		28.8
26		65.8		64.9		22.6		22.9		22.9		22.9		22.9
27		16.8		108.6		23.2		23.0		23.0		23.0		23.0
	Glc	1′ 102.3	*Glc*	1′ 102.3	*Glc*	1′ 102.3	*Glc*	1′ 102.3	*Glc*	1′ 102.4	*Xyl*	1′ 99.9	*Xyl*	1′ 100.7
		2′ 75.4		2′ 75.4		2′ 75.4		2′ 75.4		2′ 75.2		2′ 74.9		2′ 75.6
		3′ 76.6		3′ 76.5		3′ 76.6		3′ 76.6		3′ 78.4		3′ 75.5		3′ 88.5
		4′ 78.1		4′ 78.0		4′ 78.1		4′ 78.1		4′ 71.5		4′ 70.3		4′ 69.4
		5′ 76.9		5′ 77.0		5′ 77.0		5′ 77.0		5′ 78.3		5′ 63.0		5′ 66.4
		6′ 61.3		6′ 61.2		6′ 61.4		6′ 61.4		6′ 62.6				
	Rha	1″ 102.5	*Rha*	1″ 102.5	*Rha*	1″ 102.5	*Rha*	1″ 102.5	*Glc*	1″ 106.8	*Rha*	1″ 101.8	*Rha*	1″ 101.5
		2″ 72.4		2″ 72.4		2″ 72.5		2″ 72.5		2″ 75.5		2″ 72.1		2″ 72.3
		3″ 72.7		3″ 72.7		3″ 72.7		3″ 72.7		3″ 78.6		3″ 72.2		3″ 72.3
		4″ 73.9		4″ 73.9		4″ 73.9		4″ 73.9		4″ 71.5		4″ 73.7		4″ 74.1
		5″ 70.2		5″ 70.2		5″ 70.2		5″ 70.2		5″ 78.1		5″ 70.1		5″ 69.5
		6″ 18.4		6″ 18.4		6″ 18.5		6″ 18.5		6″ 62.7		6″ 18.9		6″ 19.2
							Glc	1‴ 106.8			*Glc*	1‴ 106.8	*Xyl*	1‴ 105.1
								2‴ 75.5				2‴ 75.5		2‴ 74.7
								3‴ 78.6				3‴ 78.6		3‴ 78.3
								4‴ 71.5				4‴ 71.5		4‴ 70.6
								5‴ 78.2				5‴ 78.0		5‴ 67.1
								6‴ 62.7				6‴ 62.7		
											Ac	170.3	*Glc*	1⁗ 106.9
												20.7		2⁗ 75.5
											Ac′	170.0		3⁗ 78.6
												20.5		4⁗ 71.5
														5⁗ 78.1
														6⁗ 62.7

3.5. Acid hydrolysis of *1*

A solution of **1** (4.2 mg) in 1.0 M HCl (dioxane–H_2O, 1:1, 3 ml) was heated at 95 °C for 1 h under Ar. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) and then passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA) using MeOH–H_2O (1:4) followed by MeOH, giving several decomposed compounds of genuine aglycone and a sugar fraction (0.8 mg). HPLC analysis of the sugar fraction under the following conditions showed the presence of L-rhamnose and D-glucose. Column, Capcell Pak NH_2 UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido); solvent, MeCN–H_2O (17:3); flow rate, 1.0 ml/min;

detection, RI and OR. R_t (min): 8.12 (L-rhamnose, negative polarity); 17.02 (D-glucose, positive polarity).

3.6. Compound 2

1β-Hydroxyspirosta-5,25(27)-dien-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**2**); colorless amorphous solid; $[\alpha]_D^{25}$ −66.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3388 (OH), 2925 and 2850 (CH), 1042; ^1H NMR (C_5D_5N): δ 5.87 (1H, *d*, *J* = 1.0 Hz, H-1″), 5.54 (1H, *br d*, *J* = 5.7 Hz, H-6), 4.99 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.79 and 4.76 (each 1H, *br s*, H_2-27), 4.51 (1H, *q*-like, *J* = 7.1 Hz, H-16), 4.43 and 4.00 (each 1H, *ABq*, *J* = 12.1 Hz, H_2-26), 3.99 (1H, *br m*, $W_{1/2}$ = 22.4 Hz, H-3), 3.68 (1H, *dd*, *J* = 11.9, 3.9 Hz, H-1), 2.81 (1H, *m*, H-2eq), 2.71 (1H, *m*, H-4eq), 2.52 (1H, *dd*, *J* = 12.0, 12.0 Hz, H-4ax), 2.16 (1H, *q*-like, *J* = 11.9 Hz, H-2ax), 1.67 (3H, *d*, *J* = 6.2 Hz, Me-6″), 1.19 (3H, *s*, Me-19), 1.02 (3H, *d*, *J* = 7.0 Hz, Me-21), 0.86 (3H, *s*, Me-18); For ^{13}C NMR (C_5D_5N) spectroscopic data, see Table 2; HRESI–TOFMS *m*/*z*: 737.4139 $[M+H]^+$ (calculated for $C_{39}H_{61}O_{13}$, 737.4112).

3.7. Acid hydrolysis of 2

Compound **2** (4.2 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone fraction and a sugar fraction (0.8 mg). The aglycone fraction was applied to a silica gel column eluted with $CHCl_3$–MeOH (19:1) to yield **2a** (1.8 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of L-rhamnose and D-glucose. R_t (min): 8.25 (L-rhamnose, negative polarity); 17.35 (D-glucose, positive polarity).

3.8. Compound 3

(22*S*)-16β,22-Dihydroxycholest-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**3**); colorless amorphous solid; $[\alpha]_D^{25}$ −38.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3375 (OH), 2935 (CH), 1060, 1036; ^1H NMR (C_5D_5N): δ 5.88 (1H, *d*, *J* = 1.4 Hz, H-1″), 5.34 (1H, *br d*, *J* = 4.9 Hz, H-6), 4.96 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.64 (1H, *ddd*, *J* = 7.5, 7.5, 4.7 Hz, H-16), 4.41 (1H, *br d*, *J* = 9.5 Hz, H-22), 3.88 (1H, *br m*, $W_{1/2}$ = 19.2 Hz, H-3), 1.69 (3H, *d*, *J* = 6.2 Hz, Me-6″), 1.25 (3H, *d*, *J* = 6.9 Hz, Me-21), 1.13 (3H, *s*, Me-18), 0.92 (3H, *s*, Me-19), 0.90 (3H × 2, *d*, *J* = 6.5 Hz, Me-26 and Me-27); For ^{13}C NMR (C_5D_5N) spectroscopic data, see Table 2; HRESI–TOFMS *m*/*z*: 727.4645 $[M+H]^+$ (calculated for $C_{39}H_{67}O_{12}$, 727.4633).

3.9. Compound 4

(22*S*)-16β-[(β-D-Glucopyranosyl)oxy]-22-hydroxycholest-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside (**4**); colorless amorphous solid; $[\alpha]_D^{25}$ −46.0 (*c* 0.10; C_5H_5N); IR ν_{max} (film) cm^{-1}: 3387 (OH), 2935 (CH), 1072, 1030; ^1H NMR (C_5D_5N): δ 5.24 (1H, *br d*, *J* = 4.7 Hz, H-6), 4.53 (1H, *ddd*, *J* = 7.7, 7.7, 4.5 Hz, H-16), 4.31 (1H, *br d*, *J* = 7.4 Hz, H-22), 3.84 (1H, *br m*, $W_{1/2}$ = 21.1 Hz, H-3), 1.19 (3H, *d*, *J* = 7.0 Hz, Me-21), 0.97 (3H, *s*, Me-18), 0.91 (3H, *d*, *J* = 6.2 Hz, Me-26), 0.90 (3H, *d*, *J* = 6.2 Hz, Me-27), 0.88 (3H, *s*, Me-19); for ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (C_5D_5N) spectroscopic data, see Table 2; HRESI–TOFMS *m*/*z*: 889.5142 $[M+H]^+$ (calculated for $C_{45}H_{77}O_{17}$, 889.5161).

3.10. Compound 5

(22*S*)-16β-[(β-D-Glucopyranosyl)oxy]-22-hydroxycholest-5-en-3β-yl β-D-glucopyranoside (**5**); colorless amorphous solid; $[\alpha]_D^{25}$ −34.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3387 (OH), 2936 (CH), 1060; ^1H NMR (C_5D_5N): δ 5.22 (1H, *br d*, *J* = 4.8 Hz, H-6), 4.53 (1H, *m*, H-16), 4.31 (1H, *m*, H-22), 3.89 (1H, *br m*, $W_{1/2}$ = 23.0 Hz, H-3), 1.18 (3H, *d*, *J* = 7.0 Hz, Me-21), 0.96 (3H, *s*, Me-18), 0.90 (3H, *d*, *J* = 6.2 Hz, Me-26), 0.89 (3H, *d*, *J* = 6.3 Hz, Me-27), 0.87 (3H, *s*, Me-19); for ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (C_5D_5N), see Table 2; HRESI–TOFMS *m*/*z*: 743.4626 $[M+H]^+$ (calculated for $C_{39}H_{67}O_{13}$, 743.4582).

3.11. Compound 6

(22*S*)-16β-[(β-D-Glucopyranosyl)oxy]-3β,22-dihydroxycholest-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-(3,4-di-*O*-acetyl-β-D-xylopyranoside) (**6**); colorless amorphous solid; $[\alpha]_D^{25}$ −64.0 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3387 (OH), 2926 (CH), 1750 (C=O), 1073, 1040; ^1H NMR (C_5D_5N): δ 5.47 (1H, *br d*, *J* = 5.4 Hz, H-6), 4.53 (1H, *m*, H-16), 4.30 (1H, *m*, H-22), 3.79 (1H, *br m*, $W_{1/2}$ = 21.4 Hz, H-3), 3.66 (1H, *dd*, *J* = 12.1, 3.7 Hz, H-1), 1.34 (3H, *s*, Me-19), 1.17 (3H, *d*, *J* = 6.9 Hz, Me-21), 1.06 (3H, *s*, Me-18), 0.89 (3H × 2, *d*, *J* = 6.2 Hz, Me-26 and Me-27); for ^1H NMR spectroscopic data of the sugar moieties, see Table 1; for ^{13}C NMR (C_5D_5N), see Table 2; HRESI–TOFMS *m*/*z*: 959.5245 $[M+H]^+$ (calculated for $C_{48}H_{79}O_{19}$, 959.5216).

3.12. Compound 7

(22*S*)-16β-[(β-D-Glucopyranosyl)oxy]-3β,22-dihydroxycholest-5-en-1β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-xylopyranoside (**7**); colorless amorphous solid; $[\alpha]_D^{25}$ −31.9 (*c* 0.10; MeOH); IR ν_{max} (film) cm^{-1}: 3385 (OH), 2921 (CH), 1074, 1041; ^1H NMR (C_5D_5N): δ 5.48 (1H, *br d*, *J* = 5.6 Hz, H-6), 4.50 (1H, *ddd*, *J* = 7.4, 7.4, 4.6 Hz, H-16), 4.29 (1H, *m*, H-22), 3.83 (1H, *br m*, $W_{1/2}$ = 20.3 Hz, H-3), 3.71 (1H, *dd*, *J* = 11.9, 3.7 Hz, H-1), 1.40 (3H, *s*, Me-19), 1.18 (3H, *d*, *J* = 7.0 Hz, Me-21), 1.05 (3H, *s*, Me-18), 0.90 (3H, *d*, *J* = 6.2 Hz, Me-26), 0.89 (3H, *d*, *J* = 6.2 Hz, Me-27); for the ^1H NMR spectroscopic data of the sugar moieties,

see Table 1; for ^{13}C NMR (C_5D_5N), see Table 2; HRESI–TOFMS *m/z*: 1007.5375 $[M+H]^+$ (calculated for $C_{49}H_{83}O_{21}$, 1007.5427).

3.13. Acid hydrolysis of 3–7

Compounds **3** (4.2 mg), **4** (4.0 mg), **5** (4.8 mg), **6** (4.2 mg), and **7** (4.4 mg) were independently subjected to acid hydrolysis as described for **2** to give aglycones (**3a**: 1.4 mg, 0.9 mg and 1.5 mg from **3**, **4** and **5**, and **6a**: 2.0 mg and 2.1 mg from **6** and **7**, respectively) and sugar fractions. HPLC analysis of the sugar fractions under the same conditions as for **1** showed the presence of L-rhamnose and D-glucose in those of **3** and **4**, D-glucose in that of **5**, and L-rhamnose, D-xylose and D-glucose in those of **6** and **7**. R_t (min): 10.61 (D-xylose, positive polarity).

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