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STEROIDAL SAPONINS FROM THE BULBS OF *LILIUM REGALE* AND *L. HENRYI*

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Key Word Index—*Lilium regale*; *L. henryi*; Liliaceae; bulbs; steroidal saponins; 3-hydroxy-3-methylglutaric acid; cyclic AMP phosphodiesterase; inhibitory activity.

Abstract—Two new and three known steroidal saponins were isolated from the fresh bulbs of *Lilium regale* and two known saponins from those of *L. henryi*. The structures of the new saponins were established by extensive spectral data, hydrolysis and chemical correlation as (25*R*)-27-*O*-[(*S*)-3-hydroxy-3-methylglutaroyl]-spirost-5-ene-3β,27-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside and (25*S*)-spirost-5-ene-3β,17α,27-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside. Inhibitory activity of the saponins on cyclic AMP phosphodiesterase was evaluated.

INTRODUCTION

The genus *Lilium* with more than 90 species is distributed throughout the northern temperate zone [1]. Bulbs of *Lilium* plants have long been used both as a food and as a medicinal agent in China and Japan [2]. Lilies are often attacked by a number of virus diseases, which sometimes cause fatal damage to the plants [3]. *Lilium regale* and *L. henryi* are known for their strong resistance to virus diseases, as well as *L. hansonii* [4]. The structural assignments of the several phenolic compounds from the bulbs of *L. regale* [5, 6] and *L. henryi* [7], which were suggested to protect the plants from viral infection, have already been discussed in earlier communications.

Recently, we have isolated a number of steroidal saponins from the bulbs of *Lilium* plants [8–15], some of which are unique in structure. There is, however, no report on the isolation of a steroidal saponin from *L. regale* and *L. henryi*. Our investigation into the saponin constituents in the bulbs has resulted in the discovery of two new and three known steroidal saponins in *L. regale*, and two known saponins in *L. henryi*. This paper provides detailed evidence consistent with the structural assignments of the new saponins as (25*R*)-27-*O*-[(*S*)-3-hydroxy-3-methylglutaroyl]-spirost-5-ene-3β,27-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside and (25*S*)-spirost-5-en-3β,17α,27-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside. Furthermore, inhibitory activity of the saponins and their derivatives on cyclic AMP phosphodiesterase is discussed.

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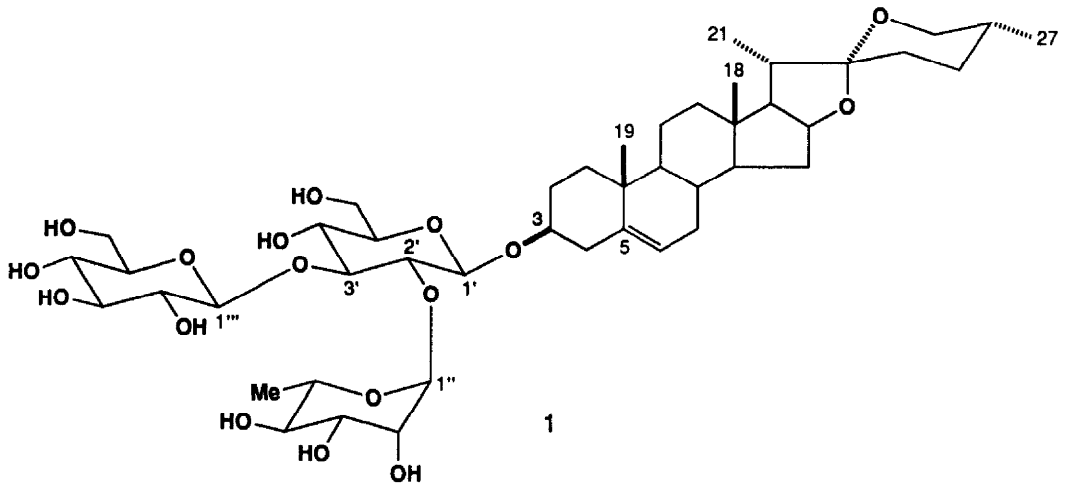
RESULTS AND DISCUSSION

The concentrated 1-butanol-soluble fraction of the methanolic bulb extract of *L. regale* was subjected to repeated silica gel, Diaion HP-20 and ODS silica gel column chromatographies to yield compounds **1**–**5**, and those of *L. henryi* to yield **3** and **6**.

Compounds **1**–**3** and **6** were identified as (25*R*)-spirost-5-en-3β-ol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (gracillin), 22-*O*-methyl-26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-ene-3β,22ξ,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (trigofoenoside D-1), (25*R*)-27-*O*-(3-hydroxy-3-methylglutaroyl) spirost-5-ene-3β,27-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (brownioside) and (25*R*)-27-*O*-(3-hydroxy-3-methylglutaroyl)-spirost-5-ene-3β,27-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside, respectively. Compound **1** was previously isolated from *Costus speciosus* (Zingiberaceae) [16], **2** from *Trigonella foenum-graecum* (Leguminosae) [17], **3** from *L. brownii* var. *brownii* (Liliaceae) [10], *L. brownii* var. *colchesteri* [11], *L. speciosum* forma *vestale* [12] and *L. mackliniae* [13], and **6** from *L. brownii* var. *colchesteri* [11] and *L. mackliniae* [13].

The absolute configuration of the 3-hydroxy-3-methylglutaroyl moiety of **3** was determined by the following method. Alkaline methanolysis of **3** with 3% sodium methoxide in methanol yielded deacylbrownioside (**3b**) [10–12] and monomethyl 3-hydroxy-3-methylglutarate (**3c**). The specific rotation of **3c** was identical to that of authentic monomethyl (*S*)-3-hydroxy-3-methylglutarate ($[\alpha]_D$ +3.0° in chloroform) derived from the partial hydrolysate of tubeimoside I [18, 19]. Furthermore, the ^{1}H NMR spectra of the (*S*)-(−)- and



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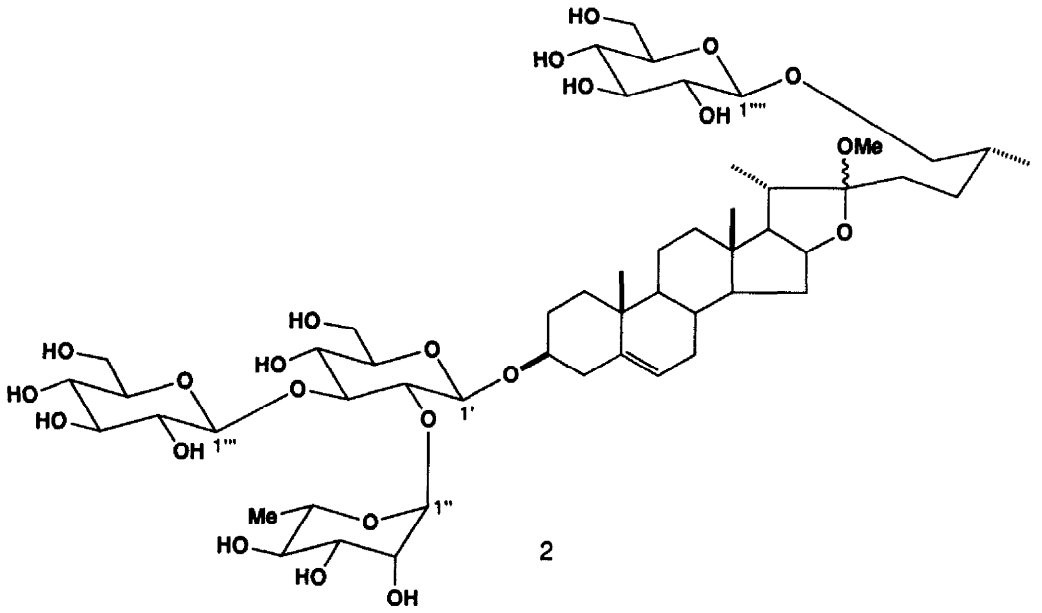

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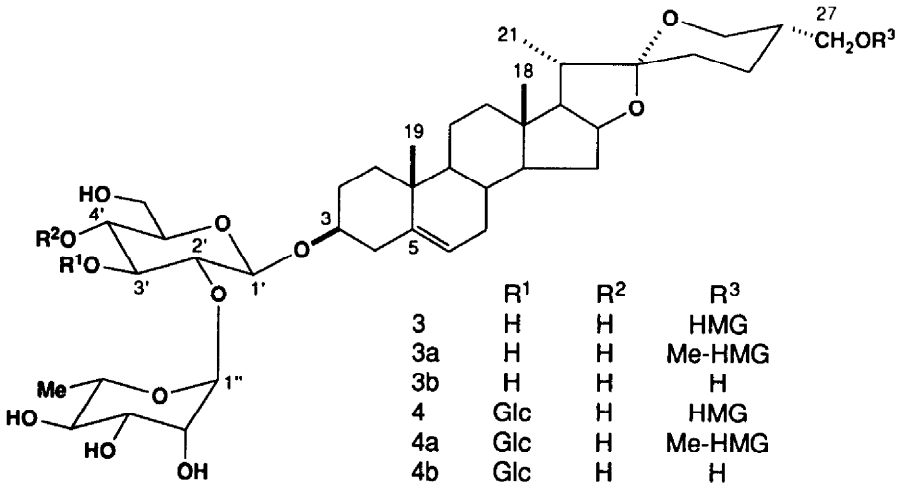
(*R*)-(+)-phenylethylamine conjugates (**3d** and **3e**) with **3c** were superimposable on those with (*S*)-(−)- and (*R*)-(+)-phenylethylamine conjugates of monomethyl (*S*)-3-hydroxy-3-methyglutarate, respectively. Thus, the absolute configuration of the 3-hydroxy-3-methylglutaroyl moiety of **3** was unequivocally determined to be *S*.

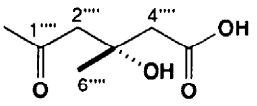
Compound **4** was obtained as needles, $C_{51}H_{80}O_{22}$ by secondary-ion (SI) mass and ^{13}C NMR spectra. On comparison of the ^{13}C NMR signals of **4** with those of **3** (Table 1), a set of additional signals, corresponding to a terminal β-D-glucopyranosyl unit appeared, and the signals due to the inner glucose moiety varied, while the signals due to the aglycone and 3-hydroxy-3-methylglutaroyl moieties remained unaffected. It was observed that the signal of the C-3 of the inner glucose was markedly shifted to downfield at δ89.5 as compared with that of **4**, suggesting that the C-3 hydroxyl position of the inner glucose is the glycosylated position to which the additional D-glucose is attached. Treatment of **4** with diazomethane yielded a monomethyl ester (**4a**), and alkaline methanolysis of **4** with 3% sodium methoxide yielded monomethyl (*S*)-3-hydroxy-3-methylglutarate (**3c**) and a steroidal saponin (**4b**). Mild hydrolysis of **4b** with 0.2 M hydrochloric acid in dioxane–H_2O (1:1) produced deacylbrownioside (**3b**) as one of the partial hydrolysates. Accordingly, the structure of **4** was formulated as (25*R*)-27-*O*-[(*S*)-3-hydroxy-3-methylglutaroyl]-spirost-5-ene-3β,27-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside.

Compound **5** was obtained as an amorphous powder. The SI mass spectrum showed quasimolecular ion peaks at *m/z* 1140 $[M+K+Na]^+$, 1117 $[M+K]^+$ and 1101 $[M+Na]^+$, corresponding to the molecular formula, $C_{52}H_{86}O_{23}$. Compound **5** was hydrolysed with 1 M

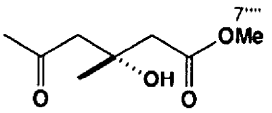


	R^1	R^2	R^3
3	H	H	HMG
3a	H	H	Me-HMG
3b	H	H	H
4	Glc	H	HMG
4a	Glc	H	Me-HMG
4b	Glc	H	H
6	H	Glc	HMG
6a	H	Glc	Me-HMG
6b	H	Glc	H

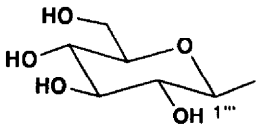
HMG:

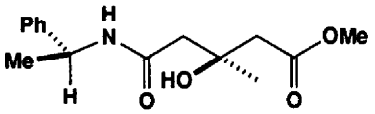


Me-HMG:

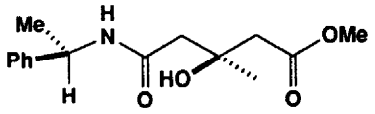


Glc:

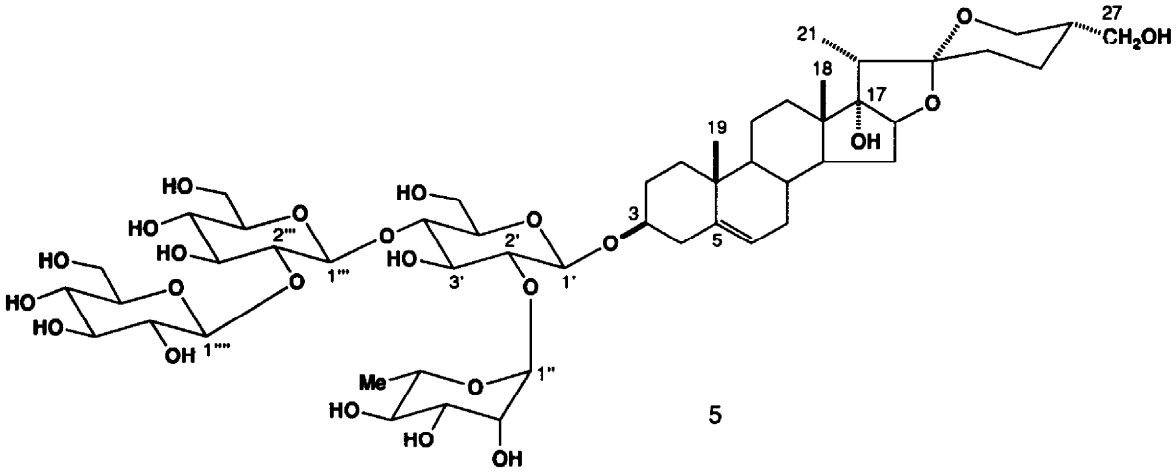




3d



3e



5

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

C	1	2	3	4	4a	4b	5	6
1	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5
2	30.0	30.1	30.2	30.1	30.1	30.1	30.1	30.2
3	77.9	77.8	78.2	77.9	77.9	77.9	78.2	78.2
4	38.7	38.7	39.0	38.6	38.8	38.8	39.0	38.9
5	140.8	140.8	140.9	140.8	140.9	140.8	140.8	140.8
6	121.9	121.8	121.7	121.8	121.8	121.9	121.8	121.8
7	32.2[a]	32.2[a]	32.2[a]	32.2[a]	32.2[a]	32.3[a]	32.5	32.2[a]
8	31.9	31.7	31.7	31.7	31.8	31.8	32.4	31.7
9	50.3	50.3	50.3	50.3	50.3	50.3	50.2	50.3
10	37.2	37.2	37.2	37.2	37.2	37.2	37.1	37.2
11	21.1	21.1	21.1	21.1	21.1	21.1	21.0	21.1
12	39.9	39.8	39.9	39.9	39.9	39.9	32.1	39.8
13	40.5	40.8	40.5	40.5	40.5	40.5	45.2	40.5
14	56.7	56.6	56.6	56.7	56.7	56.7	53.1	56.6
15	32.3[a]	32.3[a]	32.3[a]	32.3[a]	32.4[a]	32.4[a]	31.8	32.3[a]
16	81.1	81.3	81.2	81.2	81.3	81.2	90.1	81.2
17	62.9	64.2	62.8	62.8	62.9	63.0	90.2	62.8
18	16.3	16.3	16.3	16.3	16.3	16.4	17.2	16.3
19	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4
20	42.0	40.7	42.0	42.0	42.0	42.1	44.9	42.0
21	15.0	16.5	14.9	14.9	14.9	15.1	9.7	14.9
22	109.3	112.7	109.4	109.4	109.4	109.7	110.3	109.4
23	31.7	30.8	31.1	31.1	31.2	31.6	31.8	31.1
24	29.3	28.2	23.7	23.7	23.7	24.1	23.6	23.7
25	30.6	34.2	35.5	35.5	35.6	39.2	39.0	35.5
26	66.9	75.2	63.0	63.1	63.0	64.0	64.0	63.1
27	17.3	17.2	66.1	66.1	66.1	64.4	64.4	66.1
OMe		47.3						
1′	100.0	100.0	100.4	100.0	100.1	100.1	99.8	100.0
2′	77.7	77.8	79.6	77.8	77.8	77.8	77.3	77.7
3′	89.6	89.5	77.9[b]	89.5	89.6	89.6	78.2	76.2
4′	69.6	69.6	71.8	69.6	69.7	69.6	83.2	82.1
5′	77.1	77.1	78.0[b]	77.1	77.1	77.1	77.2	77.3
6′	62.5	62.4	62.7	62.5	62.5	62.5	62.5	62.0[b]
1″	102.2	102.2	102.0	102.2	102.2	102.2	101.8	101.8
2″	72.5	72.4	72.5	72.5	72.5	72.5	72.5	72.4
3″	72.8	72.8	72.8	72.8	72.8	72.8	72.8	72.8
4″	74.1	74.1	74.2	74.1	74.2	74.2	74.2	74.1
5″	69.6	69.6	69.5	69.6	69.7	69.7	69.4	69.5
6″	18.7	18.7	18.7	18.7	18.7	18.7	18.7	18.7
1‴	104.5	104.5		104.5	104.6	104.5	103.1	105.2
2‴	75.0	75.0		75.0	75.0	75.0	85.5	75.0
3‴	78.7[b]	78.6[b]		78.7[b]	78.7[b]	78.7[b]	77.7	78.3[c]
4‴	71.5	71.5		71.5	71.6	71.5	70.9	71.2
5‴	78.5[b]	78.4[b]		78.5[b]	78.6[b]	78.5[b]	78.3	78.5[c]
6‴	62.5	62.4		62.5	62.5	62.5	62.1	62.1[b]
1⁗		104.9	171.6	171.6	171.5		107.3	171.6
2⁗		75.2	46.4[c]	46.6[c]	46.3		76.7	46.4[d]
3⁗		78.6[b]	70.0	70.0	69.9		78.2	70.0
4⁗		71.8	46.5[c]	46.8[c]	46.3		70.7	46.5[d]
5⁗		78.4[b]	174.7	174.6	171.9		78.8	174.8
6⁗		62.9	28.3	28.3	28.3		62.0	28.3
7⁗					51.3			

[a–d]Assignments with the same superscript may be reversed in each column.

hydrochloric acid to afford D-glucose and L-rhamnose, which were identified by converting them to the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives followed by HPLC analysis [20–22]. The aglycone decomposed under acid conditions. The ^{1}H NMR spectrum of **5** exhibited signals due to four anomeric protons at δ6.22 (*br s*), 5.25 (*d*, J = 7.4 Hz), 5.0 (*d*, J = 7.4 Hz) and 4.96 (*d*, J = 7.7 Hz), an olefinic proton at δ5.24 (*br s*), two angular methyl protons at δ1.05 and 0.96 (each *s*), and two secondary methyl protons at δ1.74 (*d*, J = 6.1 Hz) and 1.25 (*d*, J = 7.1 Hz). The signal at δ1.74 was due to the methyl group of rhamnose. The above ^{1}H NMR spectral data and comparison of the ^{13}C NMR signals arising from the aglycone moiety of **5** with those of **1** proved **5** to be a spirost-5-en-3β-ol derivative bearing a primary hydroxyl group, a tertiary hydroxyl group and a tetrasaccharide group. The ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and Homonuclear Hartmann–Hahn (HOHAHA) spectra indicated the presence of the two partial structures in **5** (Fig. 1). The ^{13}C–^{1}H long-range correlations from the three quaternary ^{13}C signals at δ110.3, 90.2 and 45.2 observed in the HMBC spectrum of **5** are illustrated in Fig. 2. Furthermore, in the ^{13}C NMR spectrum of **5**, the signals assignable to the C-12, C-14 and C-21 were shifted to upper fields by 7.8, 3.6 and 5.3 ppm, respectively, accompanied by downfield shifts of the signals due to the C-13, C-16 and C-20 by 4.7, 9.0 and 2.9 ppm, as compared with those of **1**. The above data clearly accounted for the presence of the C-17α and C-27 hydroxyl groups. The (25*S*)-configuration of **5** was confirmed by the ^{1}H NMR parameters of the H-26 methylene protons at δ4.06 (*dd*, $J_{26eq,26ax}$ = 11.0 Hz, $J_{26eq,25ax}$ = 3.6 Hz) and 3.90 (*dd*, $J_{26ax,26eq}$ = 11.0 Hz, $J_{26ax,25ax}$ = 11.0 Hz). Thus, the structure of the aglycone moiety of **5** was shown to be (25*S*)-spirost-5-ene-3β,17α,27-triol. The structure of the tetrasaccharide moiety was determined by the following data. A combined use of the ^{1}H–^{1}H COSY and HOHAHA experiments allowed the sequential assignments of the all resonances for each monosaccharide, starting from the anomeric proton signals at δ6.22, 5.25, 5.00 and 4.96 (Table 2). A ^{1}H–^{13}C one-bond chemical shift correlation experiment via ^{1}H–^{13}C COSY spectrum correlated all proton resonances with those of the corresponding carbons. Comparison of the ^{13}C assignments with those of reference methyl glycosides [23] revealed the presence of a terminal β-D-glucopyranosyl unit (δ107.3, 76.7, 78.2, 70.7, 78.8 and 62.0), a terminal α-L-rhamnopyranosyl unit (δ101.8, 72.5, 72.8, 74.2, 69.4 and 18.7), a 2-substituted β-D-glucopyranosyl unit (δ103.1, 85.5, 77.7, 70.9, 78.3 and 62.1) and a 2,4-disubstituted β-D-glucopyranosyl unit (δ99.8, 77.3, 78.2, 83.2, 77.2 and 62.5). In the HMBC spectrum, the anomeric proton signals at δ6.22 (terminal rhamnose), 5.25 (terminal glucose), 5.0 (2-substituted glucose) and 4.96 (2,4-disubstituted glucose) showed correlations with the carbon signals at δ77.3 (C-2 of the 2,4-disubstituted glucose), 85.5 (C-2 of the 2-substituted glucose), 83.2 (C-4 of the 2,4-disubstituted glucose) and 78.2 (C-3 of the aglycone), respectively (Fig. 3). Accordingly, the structure of the tetrasaccharide moiety was

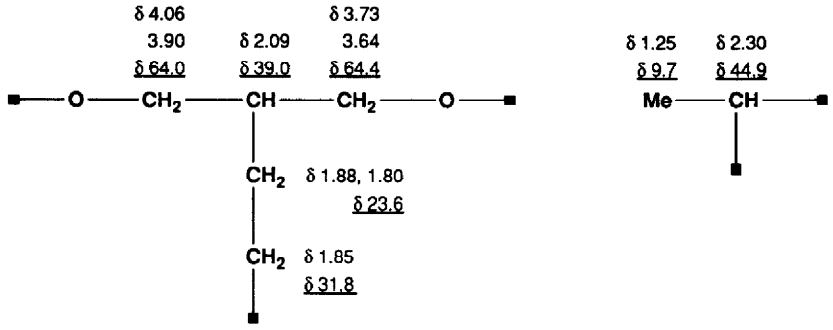


Fig. 1. ^{1}H and ^{13}C NMR chemical shifts of the partial structures of **5** in pyridine-d_5.

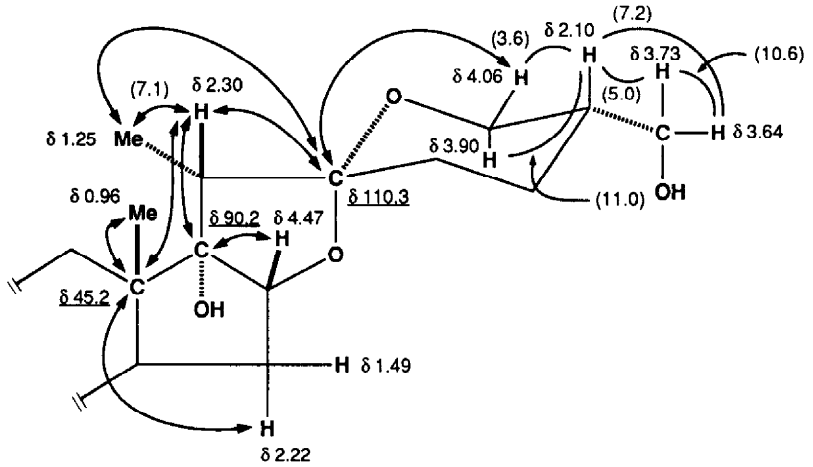


Fig. 2. ^{1}H-^{13}C long-range correlations of the aglycone moiety of **5** in pyridine-d_5. *J* vlaues (Hz) in the ^{1}H NMR spectrum are given in parentheses. Underlined figures indicate ^{13}C NMR chemical shifts.

established as *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)]-β-D-glucopyranose, and the full structure of **5** as (25*S*)-spirost-5-ene-3β,17α,27-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside. Compounds **4** and **5** are new steroidal saponins.

The inhibitory activity of the saponins and their derivatives on cyclic-AMP phosphodiesterase was examined [24, 25]. The 50% inhibitory concentrations (IC_{50}) are shown in Table 3. Compounds **1**, **3**, **4** and **6** showed considerable inhibitory activity (ref. papaverine: IC_{50} 3.0×10^{-5} M). Methylation of the 3-hydroxy-3-methylglutaroyl moiety of **3**, **4** and **6** apparently reduced their inhibitory effects (**3a** [10], **4a** and **6a** [11]). 27-Hydroxyspirostanol saponins without 3-hydroxy-3-methylglutaroyl group at the C-27 hydroxyl position (**3b** [10], **4b** and **6b** [11]), and the corresponding furostanol saponin (**2**) of **1** showed no activity.

EXPERIMENTAL

General. Mps: uncorr; NMR (ppm, *J* Hz): 1D (Bruker AM-400) and 2D (Bruker AM-500); CC: silica gel (Fuji-Davison), ODS (Nacalai Tesque) and Diaion HP-20 (Mitsubishi-kasei); TLC: precoated Kieselgel 60 F_{254} (0.25 mm or 0.5 mm thick, Merck) and RP-18 $F_{254}S$ (0.25 mm thick, Merck). HPLC: a Tosoh HPLC system (pump, CCPM; controller, CCP controller PX-8010; detector, RI-8010 or UV-8000). Tubeimoside I and its partial hydrolysate were obtained through the courtesy of Dr R. Kasai of the Hiroshima University School of Medicine, Japan.

Extraction and isolation. Fresh bulbs of *L. regale* (1.7 kg) purchased from Yamato-nouen, Japan, were cut into pieces and extracted with MeOH under reflux. The extract was concd to almost dryness under red. pres., and the crude residue, after dilution with H_2O, was extracted with *n*-BuOH. CC of the *n*-BuOH-soluble phase on silica gel and elution with CH_2Cl_2–MeOH with increasing proportion of MeOH (19:1, 9:1, 4:1, 2:1) gave 5 frs. Fractionation of the 4th fr. was carried out by passage through a Diaion HP-20 column with H_2O increasing amount of MeOH. The MeOH eluate fr. was chromatographed on silica gel with a gradient mixt. of $CHCl_3$–MeOH–H_2O (140:20:1, 100:20:1, 80:20:1, 70:20:1) and on ODS silica gel with MeOH–H_2O (4:1) to yield **1** (64.2 mg). The 5th fr. was further fractionated with Diaion HP-20 CC and the MeOH eluate fr. was

Table 2. ^{1}H NMR spectral data for oligosaccharide unit of compound **5** (in pyridine-d_5)

Glc 1′	4.96 *d* (7.7)
2′	4.20 *dd* (9.4, 7.7)
3′	4.28 *dd* (9.4, 9.4)
4′	3.82 *dd* (9.4, 9.4)
5′	4.14 *br dd* (9.4, 5.0)
6′a	5.02 *br d* (11.7)
6′b	4.24 *dd* (11.7, 5.0)
Rha1″	6.22 *br s*
2″	4.71 *br d* (2.7)
3″	4.56 *dd* (9.2, 2.7)
4″	4.31 *dd* (9.2, 9.2)
5″	4.91 *dq* (9.2, 6.1)
6″	1.74 *d* (6.1)
Glc 1‴	5.00 *d* (7.4)
2‴	4.06 *dd* (9.2, 7.4)
3‴	4.27 *dd* (9.2, 9.2)
4‴	4.19 *dd* (9.2, 9.2)
5‴	3.94 *m*
6‴a	4.47 *br d* (11.5)
6‴b	4.27 overlapping
Glc 1⁗	5.25 *d* (7.4)
2⁗	4.13 *dd* (9.0, 7.4)
3⁗	4.20 *dd* (9.0, 9.0)
4⁗	4.30 *dd* (9.0, 9.0)
5⁗	3.86 *br dd* (9.0, 3.6)
6⁗a	4.48 *br d* (11.8)
6⁗b	4.37 *dd* (11.8, 3.6)

J values in parentheses are expressed in Hz.

subjected to silica gel CC with $CHCl_3$–MeOH–H_2O (60:20:1) to collect 5 frs (5a–e). Fr. 5a was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O–25% NH_3 soln (160:40:2:1, 120:40:2:1) and then with $CHCl_3$–MeOH–H_2O (60:20:1) to yield **3** (109 mg). Compound **4** (98.9 mg) was obtained from fr. 5c by the same procedure as for **3**. Fr. 5d was purified by ODS silica gel CC with MeOH–H_2O (13:7) to give **5** (65.4 mg). Fr. 5e was chromatographed on silica gel with a gradient mixt. of $CHCl_3$–MeOH–H_2O (60:20:1, 50:20:1, 40:20:1) and on ODS silica gel with MeOH–H_2O (7:3) to yield **2** (282 mg).

Fresh bulbs of *L. henryi* (2.3 kg) purchased from Sakata, Japan, were extracted with hot MeOH. The *n*-BuOH-soluble phase of the extract gave **3** (144 mg) and **6** (186 mg) after similar chromatographic separations as for *L. regale*.

Alkaline methanolysis of compound **3**. Compound **3** (171 mg) was treated with 3% NaOMe in MeOH at room temp. for 1 hr. The reaction mixt. was neutralized by passing it through an Amberlite IR-120B (Organo) column and evaporated under red. pres. The residue was dissolved in MeOH and the ppt. filtered off. The ppt. was identified as deacylbrownioside (**3b**) (70.0 mg). The filtrate was subjected to prep. TLC with $CHCl_3$–MeOH–H_2O–HOAc (200:40:2:1) to yield monomethyl (*S*)-3-hydroxy-3-methylglutarate (**3c**) (15.0 mg) as a viscous syrup, $[\alpha]_D^{25}$ +3.2° ($CHCl_3$; *c* 0.75); IR ν_{max}^{film} cm^{-1}: 3452 (OH), 2957, 2925 and 2854 (CH), 1718 (C=O), 1439, 1377, 1349, 1260, 1204, 1116, 1093, 1015, 979, 895, 802; ^{1}H NMR (chloroform-d_1): δ3.73 (3H, *s*, OMe), 2.72 and 2.65 (each 1H, AB*q*, J=15.6 Hz, CH_2), 2.71 and 2.63 (each 1H, AB*q*, J=15.7 Hz, CH_2), 1.40 (3H, *s*, Me).

Preparation of (S)-(−)-*phenylethylamine conjugate with compound* **3c** (**3d**). Compound **3c** (6.0 mg) was dissolved in CH_2Cl_2 (2 ml) with dicyclohexylcarbodiimide (DCC) (20 mg). After being set aside 15 min at 0°, a large excess of (*S*)-(−)-phenylethylamine was added to the soln, and the mixture stirred at room temp. for 30 min. The reaction mixture was washed with H_2O (2 ml × 3), dried over Na_2SO_4, and purified by silica gel CC with $CHCl_3$–EtOAc (3:1) to the amide (**3d**) (1.9 mg) as a viscous syrup, $[\alpha]_D^{26}$ −32.0° ($CHCl_3$; *c* 0.15); ^{1}H NMR ($CDCl_3$): δ7.37–7.23 (5H, aromatic protons), 6.51 (1H, *br d*, J=6.9 Hz, NH), 5.14 (1H, *dq*, J=6.9, 6.9 Hz, MeCHNH), 3.70 (3H, *s*, OMe), 2.58 and 2.54 (each 1H, AB*q*, J=15.3 Hz, CH_2), 2.55 and 2.42 (each 1H, AB*q*, *J*

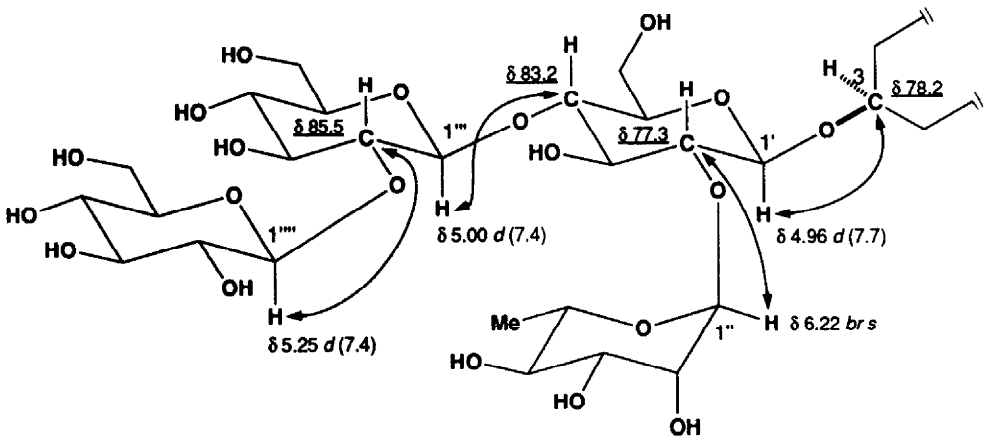


Fig. 3. ^{1}H-^{13}C long-range correlations of the saccharide moiety of **5** in pyridine-d_5. *J* values (Hz) in the ^{1}H NMR spectrum are given in parentheses. Underlined figures indicate ^{13}C NMR chemical shifts.

Table 3. Inhibitory activity on cyclic AMP phosphodiesterase of compounds **1–3**, **3a**, **3b**, **4**, **4a**, **4b**, **5**, **6**, **6a** and **6b**

	IC_{50} ($\times 10^{-5}$ M)
1	6.1
2	—
3	2.9
3a	24.0
3b	—
4	2.2
4a	71.3
4b	—
5	—
6	3.1
6a	287
6b	—

= 14.6 Hz, CH_2), 1.50 (3H, *d*, *J* = 6.9 Hz, MeCH), 1.32 (3H, *s*, Me).

Preparation of (R)-(+)-*phenylethylamine conjugate with compound* **3c** (**3e**). The amide (**3e**) (1.7 mg) was prepared from **3c** according to the method described for the preparation of **3d**. Compound **3e**: viscous syrup, $[\alpha]_D^{26}$ +43.5° ($CHCl_3$; *c* 0.17); ^{1}H NMR ($CDCl_3$): δ 7.37–7.22 (5H, aromatic protons), 6.48 (1H, *br d*, *J* = 6.9 Hz, NH), 5.13 (1H, *dq*, *J* = 6.9, 6.9 Hz, MeCHNH), 3.68 (3H, *s*, OMe), 2.58 and 2.48 (each 1H, AB*q*, *J* = 15.4 Hz, CH_2), 2.57 and 2.41 (each 1H, AB*q*, *J* = 14.5 Hz, CH_2), 1.49 (3H, *d*, *J* = 6.9 Hz, MeCH), 1.29 (3H, *s*, Me).

Compound **4**. Needles ($CHCl_3$–MeOH), mp 231–235°, $[\alpha]_D^{28}$ −52.7° (MeOH; *c* 0.32); SI-MS *m/z* 1083 $[M+K]^+$; IR ν_{max}^{KBr} cm^{-1}: 3415 (OH), 2940 (CH), 1725 (C=O), 1445, 1375, 1245, 1175, 1135, 1060, 1040, 955, 910, 835, 805, 695; ^{1}H NMR (pyridine-d_5): δ 6.36 (1H, *br s*, H-1″), 5.35 (1H, *br d*, *J* = 4.2 Hz, H-6), 5.10 (1H, *d*, *J* = 7.8 Hz, H-1‴), 4.94 (1H, *d*, *J* = 7.2 Hz, H-1′), 4.50 (1H, *q*-like, *J* = 7.6 Hz, H-16), 3.89 (1H, *dd*, *J* = 11.2, 4.0 Hz, H-26a), 3.71 (1H, *dd*, *J* = 11.2, 11.2 Hz, H-26b), 3.15 and 3.10 (each 1H, AB*q*, *J* = 15.2 Hz, H-2⁗), 3.11 and 3.07 (each 1H, AB*q*, *J* = 14.5 Hz, H-4⁗), 1.75 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.74 (3H, *s*, H-6⁗), 1.12 (3H, *d*, *J* = 6.9 Hz, H-21), 1.07 (3H, *s*, H-19), 0.82 (3H, *s*, H-18).

Methylation of compound **4** *with* CH_2N_2. Compound **4** (10.0 mg) was dissolved in MeOH and cooled at 0°. The CH_2N_2 in Et_2O was added to the sample soln. After being set aside for 2 hr at 0°, the reaction mixt. was evapd under red. pres. and the crude residue subjected to prep. TLC with $CHCl_3$–MeOH–H_2O (20:10:1) to yield the methyl ester (**4a**) (6.0 mg) of **4**. Compound **4a**: needles ($CHCl_3$–MeOH), mp 265–266.5°, $[\alpha]_D^{26}$ −63.8° ($CHCl_3$–MeOH, 2:1; *c* 0.90); negative-ion FAB-MS *m/z* 1058 $[M]^-$; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2945 (CH), 1730 (C=O), 1450, 1435, 1375, 1340, 1200, 1150, 1060, 1045, 960, 910, 835, 810; ^{1}H NMR (pyridine-d_5): δ 6.38 (1H, *br s*, H-1″), 5.35 (1H, *br d*, *J* = 4.3 Hz, H-6), 5.11 (1H, *d*, *J* = 7.8 Hz, H-1‴), 4.95 (1H, *d*, *J* = 7.0 Hz, H-1′), 4.51 (1H, *q*-like, *J* = 7.4 Hz, H-16), 3.86 (1H, *dd*, *J* = 11.1, 4.1 Hz, H-26a), 3.71 (1H, *dd*, *J* = 11.1, 11.1 Hz, H-26b), 3.63 (3H, *s*, OMe), 3.05 and 3.01 (each 1H, AB*q*, *J* = 14.8 Hz, H-2⁗), 3.03 (2H, *s*, H-4⁗), 1.76 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.69 (3H, *s*, H-6⁗), 1.12 (3H, *d*, *J* = 6.9 Hz, H-21), 1.07 (3H, *s*, H-19), 0.82 (3H, *s*, H-18).

Alkaline methanolysis of compound **4**. Compound **4** (61.7 mg) was treated with 3% NaOMe in MeOH at room temp. for 1 hr as for **3** to yield **3c** (5.2 mg) and the deacylsaponin (**4b**) (39.0 mg). Compound **4b**: amorphous powder, $[\alpha]_D^{24}$ −74.0° (pyridine; *c* 0.50); SI-MS *m/z* 924 $[M+Na+H]^+$, 901 $[M+H]^+$; IR ν_{max}^{KBr} cm^{-1}: 3415 (OH), 2940 (CH), 1445, 1375, 1245, 1060, 1040, 955, 905, 830, 805, 695; ^{1}H NMR (pyridine-d_5): δ 6.36 (1H, *br s*, H-1″), 5.34 (1H, *br d*, *J* = 3.9 Hz, H-6), 5.10 (1H, *d*, *J* = 7.8 Hz, H-1‴), 4.94 (1H, *d*, *J* = 6.7 Hz, H-1′), 3.73 (1H, *dd*, *J* = 10.7, 5.2 Hz, H-27a), 3.65 (1H, *dd*, *J* = 10.7, 7.2 Hz, H-27b), 1.75 (3H, *d*, *J* = 6.1 Hz, H-6″), 1.16 (3H, *d*, *J* = 6.9 Hz, H-21), 1.07 (3H, *s*, H-19), 0.85 (3H, *s*, H-18).

Partial hydrolysis of compound **4b**. Compound **4b** (2.9 mg) was refluxed with 0.2 M HCl in dioxane–H_2O (1:1) for 1 hr. After cooling, the reaction mixt. was neutralized with 1 M NaOH and passed through a Sep-Pak C_{18} cartridge (Waters) successively with H_2O–MeOH (4:1) and H_2O–MeOH (1:9). Deacylbrownioside (**3b**) was detected in the H_2O–MeOH (1:9) eluate by TLC and HPLC. TLC: R_f 0.61 ($CHCl_3$–MeOH–H_2O, 20:10:1); HPLC: R_t 12.5 min (column: Kaseisorb LC ODS-120-5, 4.6 mm i.d. × 250 mm, ODS, 5 μm; solvent: MeOH–H_2O, 23:2; flow rate: 0.35 ml min^{-1}; detection: RI).

Compound **5**. An amorphous powder, $[\alpha]_D^{28}$ −68.1° (MeOH; *c* 0.18); SI-MS *m/z* 1140 $[M+K+Na]^+$, 1117 $[M+K]^+$, 1101 $[M+Na]^+$; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2930 (CH), 1445, 1375, 1060, 900, 830, 805, 695; ^{1}H NMR (pyridine-d_5): δ 5.24 (1H, *br s*, H-6), 3.81 (1H, *m*, H-3), 1.25 (3H, *d*, *J* = 7.1 Hz, H-21), 1.05 (3H, *s*, H-19), 0.96 (3H, *s*, H-18). Signals for the saccharide moiety are shown in Table 2.

Acid hydrolysis of compound **5** *and determination of the absolute configurations of sugars*. A soln of **5** (5.0 mg) in 1 M HCl (dioxane–H_2O, 1:1) (2 ml) was heated in a sealed-tube at 100° for 1 hr under a N_2 atmosphere. After cooling, the reaction mixt. was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column. A Sep-Pak C_{18} cartridge was utilized to fractionate the reaction mixt. into the sugar fr. and the sapogenin fr. with H_2O–MeOH (9:1) followed by H_2O–MeOH (1:9) as the eluents. The genuine aglycone could not be detected in the sapogenin fr. The sugar fr. was treated with (−)-α-methylbenzylamine (10 mg) and $Na[BH_3CN]$ (20 mg) at 40° for 3 hr, followed by acetylation with Ac_2O in pyridine containing a catalytic amount of 4-(dimethylamino)pyridine. The 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides were analysed by HPLC (column: Tosoh TSK-gel Silica-60, 4.6 mm i.d. × 250 mm, silica gel, 5 μm; solvent: hexane–EtOH, 19:1; flow rate: 0.80 ml min^{-1}; detection: UV 230 nm). R_t (min): L-rhamnose derivative, 32.2; D-glucose derivative, 50.8.

Assay of cyclic AMP phosphodiesterase activity. The phosphodiesterase activity was assayed by the modification of the method of Thompson and Brooker as described in the previous paper [24].

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