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STEROIDAL SAPONINS FROM THE BULBS OF *LILIUM BROWNII*

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Key Word Index—*Lilium brownii*; Liliaceae; bulbs; 27-hydroxyspirostanol saponin; brownioside; isonarthogenin; 3-hydroxy-3-methylglutaric acid, furospirostanol saponin; isonuatigenin; steroidal saponins.

Abstract—The methanol extract of the fresh bulbs of *Lilium brownii* has yielded a 27-acyloxyspirostanol saponin, named brownioside and a furospirostanol saponin, which appear to be new constituents. The respective structures have been shown by the spectroscopic evidence, and alkaline- and acid-catalysed degradations to be (25*R*)-27-*O*-(3-hydroxy-3-methylglutaroyl)-spirost-5-en-3β,27-diol 3-*O*-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside and (22*S*,25*S*)-26-*O*-β-D-glucopyranosyl-22,25-epoxyfurost-5-en-3β, 26-diol 3-*O*-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside. Several known phenolic compounds have also been isolated and identified.

INTRODUCTION

Liliaceae plants are known as rich sources of steroidal saponins [1, 2]. Previously, we have isolated seven new steroidal saponins from the fresh bulbs of *Lilium pardarinum* [3]. As we pointed out in the preceding papers [3, 4], pardarinosides were the first example of the steroidal saponins isolated from the genus *Lilium*. As part of our continuous program of the chemical investigation of the *Lilium* plants, we have now examined the fresh bulbs of *Lilium brownii*, which is a common lily in China and can be found throughout the country. The bulbs are rich in starch and employed as medicine [5], but no systematic chemical work appears to have been done on the plant. From the fresh bulbs of *L. brownii*, two new steroidal saponins have been isolated together with the already mentioned phenolic constituents. This paper provides detailed evidence which is consistent with the structure assignments of the saponins as (25*R*)-27-*O*-(3-hydroxy-3-methylglutaroyl)-spirost-5-en-3β,27-diol 3-*O*-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside and (22*S*,25*S*)-26-*O*-β-D-glucopyranosyl-22,25-epoxyfurost-5-en-3β,26-diol 3-*O*-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside, respectively.

RESULTS AND DISCUSSION

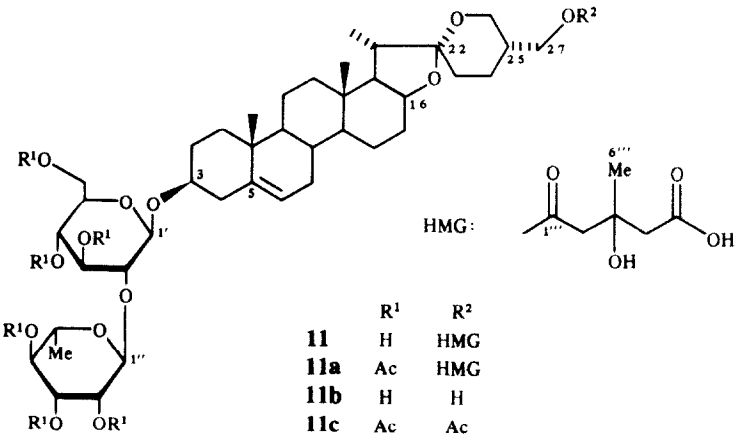
The methanolic extract of the fresh bulbs of *L. brownii* was partitioned with organic solvents. The chloroform and *n*-butanol soluble fractions were purified through the combined use of repeated silica gel, Sephadex LH-20 column chromatographies, and finally preparative TLC. The chloroform phase contained phenolic glycerides (**1**–**4**) and the *n*-butanol phase contained phenolic glycerides (**5**, **6**), phenolic glycosides (**7**–**10**) and steroidal saponins (**11**, **12**).

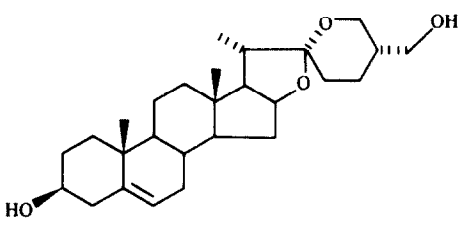
Compounds **1**–**10** were known constituents, and by the spectroscopic data and direct TLC comparison with authentic samples [6–9], the structures were identified as follows; 1,3-*O*-diferuloylglycerol (**1**), 1,2-*O*-diferuloylglycerol (**2**), 1-*O*-feruloyl-3-*O*-*p*-coumaroylglycerol (**3**), a mixture of 1-*O*-feruloyl-2-*O*-*p*-coumaroylglycerol and 1-*O*-*p*-coumaroyl-2-*O*-feruloylglycerol (**4**), 1-*O*-feruloylglycerol (**5**), 1-*O*-*p*-coumaroylglycerol (**6**), 3,6′-*O*-diferuloylsucrose (**7**), regaloside A (**8**), regaloside B (**9**), and regaloside D (**10**).

Compound **11** was obtained as needles recrystallized from a mixed solution of chloroform and methanol, mp 197.0–199.5°. The secondary ion mass spectrum (SIMS) gave, upon addition Na^+, an $[M+Na]^+$ peak at *m/z* 905, confirming the molecular weight as 882. The IR spectrum showed strong absorptions due to hydroxyl(s) (3420 cm^{-1}) and carbonyl(s) (1725 cm^{-1}). Acetylation with acetic anhydride in pyridine gave the corresponding hexaacetate (**11a**). The ^1H NMR spectrum of **11** exhibited signals attributed to anomeric protons [δ6.40 (*br s*) and 5.06 (*d*, *J* = 7.2 Hz)], an olefinic proton [δ5.32 (*br d*, *J* = 4.6 Hz)], two angular methyl protons (δ1.07 and 0.82) and secondary methyl protons [δ1.12 (*d*, *J* = 6.9 Hz)]. The ^{13}C NMR spectrum showed a total of 27 carbons arising from the aglycone moiety. Further, a quaternary carbon signal at δ109.4 with oxygen atoms and olefinic carbon signals at δ140.9 and 121.7 proved **11** to possess a Δ^5-spirostanol skeleton. Four ^{13}C NMR signals at δ19.4, 18.7, 16.3 and 14.9, which appeared as the positive signals in the 13C DEPT (135°) spectrum, were assigned to the C-19, rhamnose C-6, C-18 and C-21 positions, respectively. Thus, the structure of **11** seemed to be essentially analogous to that of diosgenin glycoside [10]. However, lack of the C-27 methyl group and the presence of an oxymethyl group were easily recognizable in the spectral data. In addition, the ^{13}C NMR spectrum showed the presence of a six carbon atom substituent, the signals of these carbons being a methyl (δ28.3), two methylene groups (δ46.5 and 46.4), a carbonyl (δ174.7), an ester carbonyl (δ171.6) and a quaternary carbon with a hydroxyl function (δ70.0). Signals in the ^1H NMR spectrum that could be ascribed to these substituents included two isolated spin systems of two methylene groups [δ3.16 and 3.12 (each 1H, AB*q*, *J* = 14.4 Hz), and 3.19 (2H, *s*)] and a singlet signal of a methyl group at δ1.78. These data showed that 3-hydroxy-3-methylglutaric acid was a

candidate for the substituent. On hydrolysis with sodium hydroxide, compound **11** was cleaved to give a steroidal saponin (**11b**) and 3-hydroxy-3-methylglutaric acid. The heptaacetate (**11c**) of **11b** was readily prepared by the usual method. On the treatment with 1 M hydrochloric acid, compound **11b** liberated D-glucose and L-rhamnose as carbohydrate compounds together with a steroidal sapogenin (**11d**). The IR, ^{1}H and ^{13}C NMR, and EI mass spectra of **11d** indicated that the C-27 which was present as a methyl in diosgenin was modified to hydroxy methyl in **11d**. The sapogenin was identified as 27-hydroxydiosgenin, that is, isonarthogenin [11, 12]. Accordingly, compound **11** was concluded to compose of the three units, 27-hydroxydiosgenin, 3-hydroxy-3-methylglutaric acid and disaccharide. The ^{13}C NMR spectrum allowed easy recognition of the sugar sequence, L-rhamnopyranose (1→2)-D-glucopyranose [4], and the anomeric configurations were determined as β for D-glucose and α for L-rhamnose from the spin-coupling constants in the ^{1}H NMR spectrum. The linkage positions between the substituents and the aglycone were established by the following spectral data. In the ^{13}C NMR spectrum of **11b**, the signal due to the C-27 was shifted to upper field by 2.0 ppm, whereas the signal due to the C-25 was shifted to lower field by 3.7 ppm as compared with those of compound **11**. Furthermore, the signals at δ4.08 (*dd*, J = 11.0, 5.4 Hz) and 4.00 (*dd*, J = 11.0, 7.8 Hz) in **11** were replaced by the signals at δ3.74 (*dd*, J = 10.5, 5.0 Hz) and 3.66 (*dd*, J = 10.5, 7.4 Hz) in **11b**, assignable to the H-27 methylene protons in the ^{1}H NMR spectrum. The above facts clearly accounted for the acyl moiety linkage to the C-27 hydroxyl position. Consequently, the sugar moiety was attached to the remaining hydroxyl group, the C-3 position on the sapogenin, which was supported by the ^{13}C NMR glycosidation shifts. The signal attributable to the C-3 position in **11d** was shifted to upper field by 7.0 ppm, whereas the signals due to the C-2 and C-4 were shifted to lower field as compared with those of **11b**. From the facts referred to above, compound **11** was formulated as (25*R*)-27-*O*-(3-hydroxy-3-methylglutaroyl)-spirost-5-en-3β,27-diol 3-*O*-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside.

Compound **12** was obtained as a white amorphous powder. The spectral features of **12** were also suggestive of a steroidal saponin. The ^{1}H NMR spectrum showed signals for three tertiary methyl groups at δ1.40, 1.05 and 0.86, and a secondary methyl group at δ1.08 (*d*, J = 6.9 Hz). In the ^{13}C NMR spectrum, the C-22 carbon of the aglycone resonated at δ120.2, which was characteristic of the furospirostanol sapogenins [10]. Further, compound **12** exhibited three peaks (δ105.5, 102.1 and 100.4) due to the anomeric carbons of the sugar moieties, and the signals ascribable to the aglycone moiety were in good accordance to those of aculeatiside A and B isolated from the root of *Solanum aculeatissimum* [13]. Aculeatisides are nuatigenin 3,26-bisdesmosides. On acid hydrolysis, compound **12** gave L-rhamnose, D-glucose and isonuatigenin (**12a**) which was produced as an artifact from nuatigenin in the acidic conditions [14]. The ^{13}C NMR spectrum verified that **12** was constituted of three units, nuatigenin, β-D-glucopyranose and α-L-rhamnopyranosyl(1→2)-β-D-glucopyranose. The two possible structures, 26-*O*-β-D-glucopyranosylnuatigenin 3-*O*-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside and 26-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl]nuatigenin 3-*O*-β-D-glucopyranoside were considered. This question was solved by the NOE difference spectrum of **12**-decaacetate (**12b**). Effects could be observed between the H-3 proton (δ3.71, *br m*) and the anomeric proton (δ4.26, *d*, J = 7.7 Hz) of α-L-rhamnose(1→2)-β-D-glucose, and between the H-26b proton (δ3.63, *d*, J = 9.5 Hz) and the anomeric proton





11d

Table 1. ^{13}C NMR spectral data for compounds **11**, **11b**, **11d** and **12**

C	11	11b	11d	12
1	37.5	37.5	37.8	37.5
2	30.2	30.2	32.6	30.2
3	78.2	78.3	71.3	78.2
4	39.0	39.0	43.5	39.0
5	140.9	140.9	142.0	140.9
6	121.7	121.7	121.0	121.8
7	32.3[a]	32.3	32.3[a]	32.2[a]
8	31.7	31.7	31.9	31.7
9	50.3	50.3	50.3	50.3
10	37.2	37.2	37.1	37.2
11	21.1	21.1	21.2	21.1
12	39.9	39.9	40.0	39.9
13	40.5	40.5	40.5	40.5
14	56.6	56.7	56.8	56.5
15	32.2[a]	32.3	32.2[a]	32.3[a]
16	81.2	81.2	81.2	81.0
17	62.8	63.0	63.0	62.5
18	16.3	16.3	16.4	16.2
19	19.4	19.4	19.6	19.4
20	42.0	42.1	42.1	38.6
21	14.9	15.1	15.1	15.2
22	109.4	109.7	109.7	120.2
23	31.1	31.6	31.6	33.1[b]
24	23.7	24.1	24.1	34.0[b]
25	35.5	39.2	29.2	83.9
26	63.0	64.4[a]	64.4[b]	77.5
27	66.1	64.1[a]	64.1[b]	24.4
1′	100.4	100.4		100.4
2′	79.6	79.7		79.7
3′	77.9[b]	77.9[b]		77.9[c]
4′	71.8	71.9		71.9
5′	78.0[b]	78.0[b]		78.0[c]
6′	62.7	62.7		62.7
1″	102.0	102.1		102.1
2″	72.5	72.6		72.6
3″	72.8	72.9		72.9
4″	74.2	74.2		74.2
5″	69.5	69.5		69.5
6″	18.7	18.7		18.7
1‴	171.6			105.5
2‴	46.5[c]			75.4
3‴	70.0			78.4[d]
4‴	46.4[c]			71.7
5‴	174.7			78.5[d]
6‴	28.3			62.7

Spectra were measured in pyridine-d_5.

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

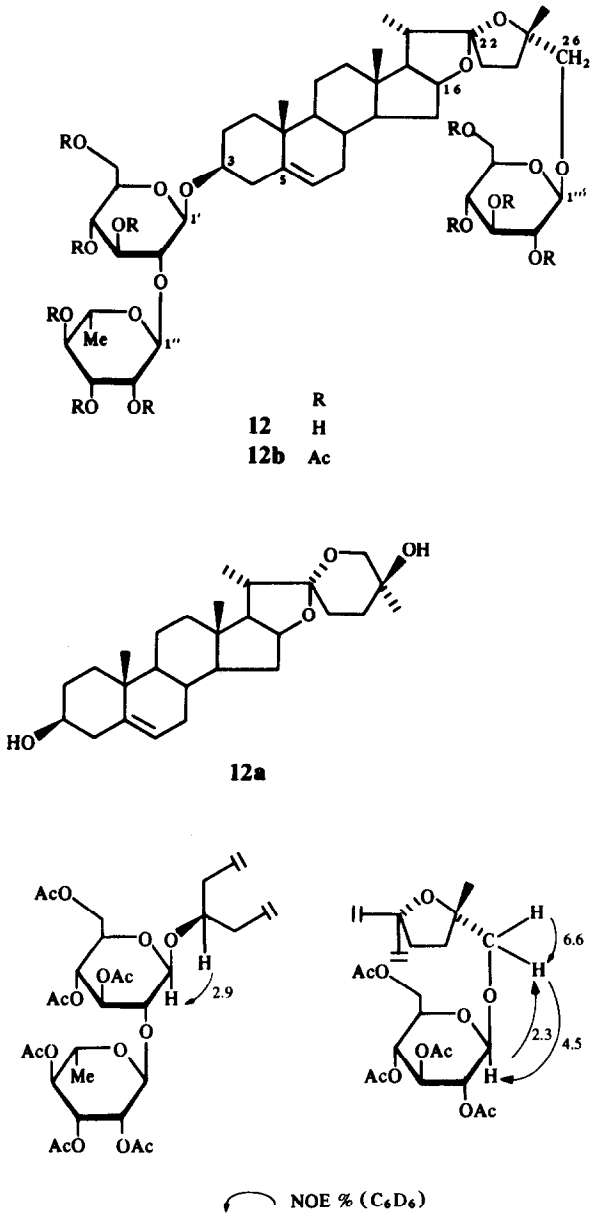


Fig. 1.

(δ4.48, *d*, J = 7.8 Hz) of β-D-glucose as shown in Fig. 1. Finally, all the available data identified the structure of compound **12** as (22*S*,25*S*)-26-*O*-β-D-glucopyranosyl-22,25-epoxyfurost-5-en-3β,26-diol 3-*O*-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside.

27-Hydroxyspirostanol sapogenins are rare in nature [11, 12, 15–18], and most of them occur as minor components accompanied by the major steroidal sapogenins. Brownioside (**11**) is a representative of a rare type of 27-hydroxyspirostanol saponin containing an acyl moiety linked at the C-27 hydroxyl position. Furospirostanol saponins are also rare in nature [13, 19, 20].

EXPERIMENTAL

General. Mps: uncorr. ^{1}H and ^{13}C NMR spectra (ppm, *J* Hz) were obtained at 400 MHz (^{1}H NMR) and 100.6 MHz (^{13}C NMR) with TMS as an int. standard. CC was carried on silica gel (Fuji Davison Co., Ltd) and on Sephadex LH-20 (Pharmacia Fine Chemicals Co., Ltd). TLC was performed on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and prep. TLC on precoated Kieselgel 60 F_{254} (0.5 mm thick, Merck). Spots were visualized under UV light (254 nm) irradiation and by spraying 10% H_2SO_4 solution followed by heating.

Extraction and isolation. The commercially available fresh bulbs of *L. brownii* (3.4 kg), after being cut into pieces, were

extracted with MeOH at 60°. The extract was evapd under vacuum nearly to dryness, and the crude residue, a dark viscous syrup, was suspended with H_2O. The suspension was extracted with $CHCl_3$, and then with *n*-BuOH. After concentration, each fraction was subjected to CC on silica gel with $CHCl_3$–EtOAc, $CHCl_3$–Me_2CO and $CHCl_3$–MeOH solvent systems for the $CHCl_3$ soluble fraction, and with $CHCl_3$–MeOH, $CHCl_3$–MeOH–H_2O, EtOAc–Me_2CO, EtOAc–MeOH and EtOAc–MeOH–H_2O for the *n*-BuOH fraction. Sephadex LH-20 CC with $CHCl_3$ or MeOH was also used for the purification. Final purification of **8** and **10** was carried out by prep. TLC with EtOAc–2-MeCOEt–MeOH–H_2O (10:10:1:1). Compounds **1** (38.9 mg), **2** (21.7 mg), **3** (33.4 mg) and **4** (3.7 mg) were isolated from the $CHCl_3$ phase, and **5** (19.7 mg), **6** (24.6 mg), **7** (33.5 mg), **8** (25.3 mg), **9** (243 mg), **10** (10.2 mg), **11** (1.01 g) and **12** (50.9 mg) from the *n*-BuOH phase.

Brownioside (**11**). Needles ($CHCl_3$–MeOH), mp 197.0 –199.5°, $[\alpha]_D^{26}$ −75.0° (MeOH; *c* 0.48). SIMS *m/z*: 905 $[M+Na]^+$. EIMS *m/z* (rel. int.): 575 (3.6), 472 (2.4), 412 (1), 355 (2.6), 342 (9.6), 300 (22), 282 (39), 270 (15), 237 (11), 197 (65), 155 (24), 128 (100), 112 (64). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2930, 2895 (CH), 1725 (C=O), 1445, 1375, 1335, 1160, 1130, 1060, 1040, 975, 960, 910, 835, 810. ^1H NMR (pyridine-d_5): δ6.40 (1H, *br s*, H-1″), 5.32 (1H, *br d*, *J* = 4.6 Hz, H-6), 5.06 (1H, *d*, *J* = 7.2 Hz, H-1′), 5.01 (1H, *dq*, *J* = 9.5, 6.4 Hz, H-5″), 4.81 (1H, *br d*, *J* = 3.3 Hz, H-2″), 4.64 (1H, *dd*, *J* = 9.3, 3.3 Hz, H-3″), 4.08 (1H, *dd*, *J* = 11.0, 5.4 Hz, H-27a), 4.00 (1H, *dd*, *J* = 11.0, 7.8 Hz, H-27b), 3.90 (1H, *m*, H-26a), 3.71 (1H, *dd*, *J* = 11.2, 11.2 Hz, H-26b), 3.19 (2H, *s*, H-4‴), 3.16, 3.12 (each 1H, AB*q*, *J* = 14.4 Hz, H-2‴), 1.79 (3H, *d*, *J* = 6.4 Hz, H-6″), 1.78 (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).

Acetylation of compound **11**. Compound **11** (25.0 mg) was acetylated with Ac_2O in pyridine. After usual work-up and chromatography on silica gel with *n*-hexane–Me_2CO (3:1), a pure acetate (**11a**) was obtained as an amorphous powder (11.2 mg). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2960 (CH), 1745 (C=O), 1440, 1365, 1255, 1220, 1130, 1075, 1030, 955, 905, 800. ^1H NMR ($CDCl_3$): δ5.39 (1H, *br d*, *J* = 4.9 Hz, H-6), 5.25 (1H, *dd*, *J* = 10.0, 3.4 Hz, H-3″), 5.24 (1H, *dd*, *J* = 9.5, 9.5 Hz, H-3′), 5.07 (1H, *dd*, *J* = 10.0, 10.0 Hz, H-4″), 5.00 (1H, *dd*, *J* = 3.4, 1.7 Hz, H-2″), 4.97 (1H, *d*, *J* = 1.7 Hz, H-1″), 4.96 (1H, *dd*, *J* = 9.5, 9.5 Hz, H-4′), 4.58 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.41 (1H, *m*, H-16), 4.39 (1H, *dq*, *J* = 10.0, 6.2 Hz, H-5″), 4.27 (1H, *dd*, *J* = 12.3, 5.0 Hz, H-6′a), 4.08 (1H, *dd*, *J* = 12.3, 2.3 Hz, H-6′b), 3.98 (1H, *dd*, *J* = 11.1, 5.2 Hz, H-27a), 3.87 (1H, *dd*, *J* = 11.1, 7.4 Hz, H-27b), 3.72 (1H, *dd*, *J* = 9.5, 7.8 Hz, H-2′), 3.69 (1H, *ddd*, *J* = 9.5, 5.0, 2.3 Hz, H-5′), 3.63 (1H, *br dd*, *J* = 11.3, 3.6 Hz, H-26a), 3.62 (1H, *m*, H-3), 3.54 (1H, *dd*, *J* = 11.3, 11.3 Hz, H-26b), 3.14, 3.09 (each 1H, AB*q*, *J* = 15.4 Hz, H-2‴ or -4‴), 3.10, 3.04 (each 1H, AB*q*, *J* = 15.0 Hz, H-2‴ or -4‴), 2.13, 2.07, 2.06, 2.02, 2.01, 1.99 (each 3H, *s*, Ac × 6), 1.66 (3H, *s*, H-6‴), 1.19 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.02 (3H, *s*, H-19), 0.97 (3H, *d*, *J* = 7.0 Hz, H-21), 0.79 (3H, *s*, H-18).

Alkaline hydrolysis of compound **11**. Hydrolysis of **11** (160 mg) with 2 M NaOH was carried out at room temp. for 2 hr. The reaction soln was neutralized with 2 M HCl and the soln was concd to give a residue, which was subjected to Sephadex LH-20 CC with MeOH and then with silica gel CC with $CHCl_3$–MeOH–25% NH_3 (100:25:2)→MeOH to give **11b** (83.9 mg) and 3-hydroxy-3-methylglutaric acid (8.1 mg). Compound **11b**, needles ($CHCl_3$–MeOH), mp 232.0–238.0°, $[\alpha]_D^{26}$ −95.7° (MeOH; *c* 0.28). Final purification of 3-hydroxy-3-methylglutaric acid was carried out by prep. TLC with $CHCl_3$–MeOH–H_2O (50:20:1). Compound **11b**, SIMS *m/z*: 739 $[M+H]^+$, 429 $[aglycone-H]^+$, 411 $[aglycone-H-H_2O]^+$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2930, 2895 (CH), 1445, 1370, 1160, 1130, 1040, 995, 965, 950, 905, 805. ^1H NMR (pyridine-d_5): δ6.39 (1H, *br s*, H-1″), 5.31 (1H, *br d*, *J* = 4.4 Hz, H-6), 5.05 (1H, *d*, *J* = 7.3 Hz, H-1′), 5.01 (1H, *dq*, *J* = 9.5, 6.2 Hz, H-5″), 4.81 (1H, *br d*, *J* = 3.2 Hz, H-2″), 4.64 (1H, *dd*, *J* = 9.3, 3.2 Hz, H-3″), 4.57 (1H, *m*, H-16), 3.74 (1H, *dd*, *J* = 10.5, 5.0 Hz, H-27a), 3.66 (1H, *dd*, *J* = 10.5, 7.4 Hz, H-27b), 1.78 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.17 (3H, *d*, *J* = 6.9 Hz, H-21), 1.07 (3H, *s*, H-19), 0.84 (3H, *s*, H-18). 3-Hydroxy-3-methylglutaric acid was identified by the ^1H, and ^{13}C NMR spectra measured in CD_3OD.

Acetylation of compound **11b**. Compound **11b** (12.6 mg) was treated with Ac_2O in pyridine and the crude acetate was purified by silica gel CC (*n*-hexane–Me_2CO, 2:1) to give a heptaacetate (**11c**) (16.5 mg). Needles (EtOH), mp 173.0–176.5. IR ν_{max}^{KBr} cm^{-1}: 2940, 2900 (CH), 1745 (C=O), 1445, 1365, 1240, 1220, 1170, 1135, 1070, 1030, 970, 955, 905. ^1H NMR ($CDCl_3$): δ5.40 (1H, *br d*, *J* = 4.9 Hz, H-6), 5.26 (1H, *dd*, *J* = 10.0, 3.4 Hz, H-3″), 5.25 (1H, *dd*, *J* = 9.6, 9.6 Hz, H-3′), 5.07 (1H, *dd*, *J* = 10.0, 10.0 Hz, H-4″), 5.00 (1H, *dd*, *J* = 3.4, 1.9 Hz, H-2″), 4.97 (1H, *d*, *J* = 1.9 Hz, H-1″), 4.96 (1H, *dd*, *J* = 9.6, 9.6 Hz, H-4′), 4.58 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.42 (1H, *m*, H-16), 4.39 (1H, *dq*, *J* = 10.0, 6.2 Hz, H-5″), 4.27 (1H, *dd*, *J* = 12.2, 4.9 Hz, H-6′a), 4.08 (1H, *dd*, *J* = 12.2, 2.3 Hz, H-6′b), 3.95 (1H, *dd*, *J* = 11.1, 5.3 Hz, H-27a), 3.82 (1H, *dd*, *J* = 11.1, 7.9 Hz, H-27b), 3.72 (1H, *dd*, *J* = 9.6, 7.8 Hz, H-2′), 3.64 (3H, overlapping, H-3, -26a, -5′), 3.53 (1H, *dd*, *J* = 11.2, 11.2 Hz, H-26b), 2.13, 2.07, 2.06, 2.04, 2.02, 2.01 1.99 (each 3H, *s*, Ac × 7), 1.19 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.02 (3H, *s*, H-19), 0.97 (3H, *d*, *J* = 6.9 Hz, H-21), 0.79 (3H, *s*, H-18).

Acid hydrolysis of compound **11b**. Hydrolysis of **11b** (55.0 mg) was performed by refluxing in 1 M HCl (H_2O–dioxane, 1:1) for 2 hr. The reaction mixture was neutralized with 2 M NaOH and the soln was evapd to dryness. The crude sample, after diluted with H_2O, was extracted with $CHCl_3$. The $CHCl_3$ layer was subjected to silica gel CC with $CHCl_3$–Me_2CO (19:1) to yield a pure sapogenin (**11d**) (10.5 mg) as prisms (MeOH), mp 210.0–220.0°, $[\alpha]_D^{25}$ −84.6° ($CHCl_3$–MeOH, 2:1; *c* 0.39), which was identified as isonarthogenin by the IR, EIMS, ^1H and ^{13}C NMR. The H_2O residue was purified by silica gel CC with $CHCl_3$–MeOH–H_2O (125:25:2) to provide L-rhamnose (8.4 mg) and D-glucose (3.5 mg). L-rhamnose; TLC, R_f 0.67 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). $[\alpha]_D^{24}$ +8.3° (H_2O; *c* 0.84). D-glucose; TLC, R_f 0.38 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). $[\alpha]_D^{24}$ +45.7° (H_2O; *c* 0.35).

Compound **12**. A white amorphous powder, $[\alpha]_D^{26}$ −78.8° (MeOH; *c* 0.16). SIMS *m/z*: 939 $[M+K]^+$, 923 $[M+Na]^+$. EIMS *m/z* (rel. int.): 430 (3), 399 (40), 368 (5), 342 (19), 300 (15), 282 (31), 271 (32), 241 (7), 215 (8), 195 (16), 155 (100), 128 (79), 112 (60), 103 (79). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 2935 (CH), 1455, 1380, 1260, 1135, 1080, 1045, 915, 870, 840, 820, 705. ^1H NMR (pyridine-d_5): δ6.37 (1H, *br s*, H-1″), 5.31 (1H, *br d*, *J* = 4.3 Hz, H-6), 5.04 (1H, *d*, *J* = 7.1 Hz, H-1′), 4.99 (1H, *dq*, *J* = 9.5, 6.2 Hz, H-5″), 4.96 (1H, *d*, *J* = 7.8 Hz, H-1‴), 4.80 (1H, *br d*, *J* = 3.2 Hz, H-2″), 4.71 (1H, *m*, H-16), 4.62 (1H, *dd*, *J* = 9.3, 3.2 Hz, H-3″), 1.77 (3H, *d*, *J* = 6.2, H-6″), 1.40 (3H, *s*, H-27), 1.08 (3H, *d*, *J* = 6.9 Hz, H-21), 1.05 (3H, *s*, H-19), 0.81 (3H, *s*, H-18).

Acid hydrolysis of compound **12**. Compound **12** (30.0 mg) was subjected to acid hydrolysis as for **11b**. The crude hydrolysate, after dilution with H_2O was extracted with EtOAc. Purification of the EtOAc extract by silica gel CC with $CHCl_3$–Me_2CO (20:1) gave a pure sapogenin (**12a**) (4.0 mg) as a white amorphous powder, $[\alpha]_D^{26}$ −112.0° ($CHCl_3$; *c* 0.40), which was identified as isonuatigenin by the IR and ^1H NMR spectra. L-rhamnose (2.5 mg) and D-glucose (6.0 mg) could be detected in the H_2O layer by the same method as for **11b**.

Acetylation of compound **12**. Compound **12** (17.9 mg) was acetylated with Ac_2O in pyridine. After usual work-up and chromatography on silica gel with *n*-hexane–Me_2CO (5:2) and *n*-hexane–EtOAc (1:1), a pure acetate (**12b**) was obtained as a

white amorphous powder (17.0 mg). IR ν_{max}^{KBr} cm^{-1}: 2960 (CH), 1755 (C=O), 1440, 1370, 1220, 1170, 1135, 1035, 995, 900, 835, 800, 755. ^1H NMR (C_6D_6): δ 5.82 (1H, *dd*, J = 10.0, 3.3 Hz, H-3″), 5.62 (1H, *dd*, J = 10.0, 10.0 Hz, H-4″), 5.51 (1H, *br*, H-6), 5.49 (1H, *dd*, J = 9.5, 9.5 Hz, H-3′), 5.45 (1H, *dd*, J = 9.3, 9.3 Hz, H-3‴), 5.41 (1H, *dd*, J = 3.3, 1.7 Hz, H-2″), 5.36 (1H, *dd*, J = 9.3, 7.8 Hz, H-2‴), 5.31 (1H, *d*, J = 1.7 Hz, H-1″), 5.29 (1H, *dd*, J = 9.3, 9.3 Hz, H-4‴), 5.20 (1H, *dd*, J = 9.5, 9.5 Hz, H-4′), 4.76 (1H, *dq*, J = 10.0, 6.2 Hz, H-5″), 4.69 (1H, *m*, H-16), 4.48 (1H, *d*, J = 7.8 Hz, H-1‴), 4.32 (1H, *dd*, J = 11.9, 4.1 Hz, H-6′a), 4.31 (1H, *dd*, J = 12.2, 4.3 Hz, H-6‴a), 4.26 (1H, *d*, J = 7.7 Hz, H-1′), 4.03 (1H, *dd*, J = 11.9, 2.2 Hz, H-6′b), 4.01 (1H, *dd*, J = 12.2, 2.1 Hz, H-6‴b), 3.99 (1H, *d*, J = 9.5 Hz, H-26a), 3.83 (1H, *dd*, J = 9.5, 7.7 Hz, H-2′), 3.71 (1H, *m*, H-3), 3.63 (1H, *d*, J = 9.5 Hz, H-26b), 3.31 (1H, *ddd*, J = 9.3, 4.3, 2.1 Hz, H-5‴), 3.25 (1H, *ddd*, J = 9.5, 4.1, 2.2 Hz, H-5′), 2.04, 1.86, 1.76, 1.74 × 2, 1.71, 1.69, 1.66 × 2, 1.60 (each 3H, *s*, Ac), 1.42 (3H, *d*, J = 6.2 Hz, H-6″), 1.27 (3H, *s*, H-27), 1.08 (3H, *d*, J = 6.9 Hz, H-21), 1.02 (3H, *s*, H-19), 0.84 (3H, *s*, H-18).

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