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LIPID AND STEROIDAL CONSTITUENTS OF *LILIUM AURATUM* VAR. *PLATYPHYLLUM* AND *L. TENUIFOLIUM*

YOSHIHIRO MIMAKI, YUTAKA SASHIDA* and HIROKO SHIMOMURA

Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji, Tokyo 192-03, Japan

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Key Word Index—*Lilium auratum* var. *platyphyllum*; *Lilium tenuifolium*; Liliaceae; acylated glycerol glucosides; regaloside H; regaloside I; polyhydroxylated steroidal glycosides; tenuifolioside A; tenuifolioside B; tenuifoliol.

Abstract—The chemical compounds in the bulbs of *Lilium auratum* var. *platyphyllum* and *L. tenuifolium* have been analysed as part of a systematic study of the genus *Lilium*. The structures of two new phenylpropanoid glycerol glucosides, regaloside H and I from *L. auratum* var. *platyphyllum*, and two new polyhydroxylated steroidal glycosides, tenuifolioside A and B from *L. tenuifolium* have been elucidated on the basis of the spectroscopic analysis and some chemical transformations.

INTRODUCTION

Bulbs of the genus *Lilium* have been described to have some medical uses [1, 2]. A survey of the literature, however, showed that early studies carried out on the bulbs of the genus *Lilium* had demonstrated only the occurrence of trivial acids, sterols, sugars and mucous polysaccharides. There was only one example of the relatively low M_r phenolics [3]. Therefore, our attention has been directed to the constituents of the lily bulbs. Compounds isolated by us include bitter phenylpropanoid glycosides [4–8], an antitumour alkaloid and its glucoside [8, 9], a steroidal glucoside [6], phenolic glycerides [10], and steroidal saponins [11, 12].

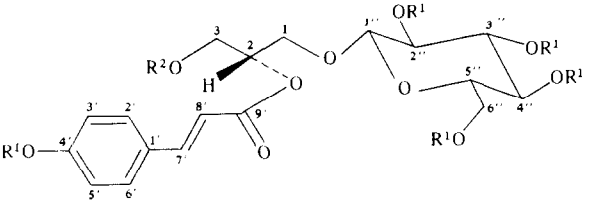
As part of our contribution to study of the genus, we have now carried out the phytochemical screening of the bulbs of *Lilium auratum* var. *platyphyllum* and *L. tenuifolium*, nothing has been published so far on their constituents.

RESULTS AND DISCUSSION

Lilium auratum var. *platyphyllum*

The concentrated chloroform and *n*-butanol soluble fractions of the methanolic bulb extract of *L. auratum* var. *platyphyllum* were subjected to repeated silica gel and Sephadex LH-20 column chromatographies, and finally preparative HPLC and preparative TLC to yield compounds **1**–**15**. Compounds **1**–**5** obtained from the chloroform-soluble phase were identified as 1,3-*O*-diferuloylglycerol, 1-*O*-feruloyl-3-*O*-*p*-coumaroylglycerol, 1,2-*O*-diferuloylglycerol, a mixture of 1-*O*-feruloyl-2-*O*-*p*-coumaroylglycerol and 1-*O*-*p*-coumaroyl-2-*O*-feruloylglycerol [10,13], and *dl*-syringaresinol [14, 15], respectively. Compounds **6**–**13** from the *n*-butanol-soluble phase were identified as 1-*O*-feruloylglycerol, 1-*O*-*p*-coumaroylglycerol [10, 16], 3,6′-*O*-diferuloylsucrose, 4-*O*-acetyl-3,6′-*O*-diferuloylsucrose, 6-*O*-acetyl-3,6′-*O*-diferuloylsucrose [4, 17], regaloside A, regaloside B [5], and regaloside D [7], respectively. The spectral characteristics of the known compounds were consistent with those previously reported.

Compound **14** was obtained from the *n*-butanol soluble-portion as a white amorphous powder with the molecular formula $C_{18}H_{24}O_{10}$ (EIMS *m/z* 400 $[M]^+$). The UV spectrum showed absorption maxima at 227, 300 sh and 313 nm, undergoing bathochromic shift (361 nm) upon addition of sodium methoxide solution. Absorptions for hydroxyl(s) (3380 cm^{-1}), a conjugated ester (1680 cm^{-1}), a double bond (1625 cm^{-1}) and an aromatic ring (1600, 1580 and 1510 cm^{-1}) were noted. The ^1H and ^{13}C NMR spectra showed that **14** consisted of *p*-coumaroyl, glycerol and β-D-glucose moieties. On acetylation with acetic anhydride in pyridine, **14** formed the corresponding hexaacetate (**14a**), the ^1H NMR spectrum of which exhibited the signals of five aliphatic and an aromatic acetyl groups. The molecular ion peak of **14a** was observed at *m/z* 652 along with the prominent fragment ion peaks at *m/z* 331, 169 and 147 in the EI mass spectrum. Alkaline treatment of **14** yielded methyl *p*-coumarate and glycerol glucoside, the latter was subsequently acetylated with acetic anhydride in pyridine to



	R^1	R^2
14	H	H
14a	Ac	Ac
15	H	Ac
15a	Ac	Ac

Table 1. ^{13}C NMR spectral data for compounds **14** and **15** in CD_3OD

C	14	15
Glycerol moiety		
1	69.0	69.0
2	74.8	71.9
3	61.8	64.3
Phenylpropanoid moiety		
1′	127.2	127.1
2′	131.2	131.3
3′	116.9	116.9
4′	161.4	161.5
5′	116.9	116.9
6′	131.2	131.3
7′	147.0	147.3
8′	115.2	114.8
9′	168.9	168.5
Glucose moiety		
1″	104.8	104.9
2″	75.1	75.1
3″	78.0	78.0[a]
4″	71.6	71.6
5″	78.0	78.1[a]
6″	62.8	62.8
Ac	—	172.6
	—	20.7

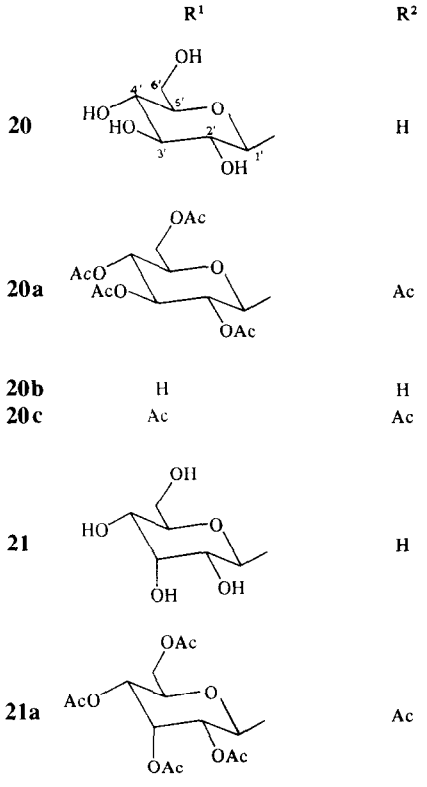
[a]Assignment may be reversed.

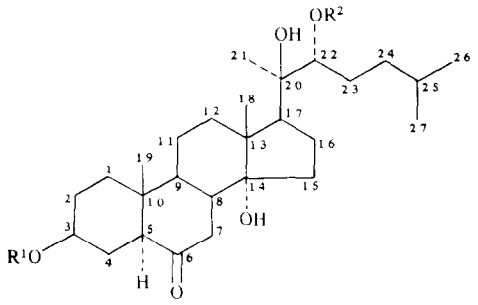
provide (2*R*)-1-*O*-β-D-glucopyranosylglycerol hexaacetate, that is, lilioside C hexaacetate (**14b**) [5, 18]. Accordingly, the fundamental structure of **14** appeared to be a *p*-coumaroyl ester of lilioside C. The ester linkage in the glycerol C-2 position was formed from *p*-coumaric acid as was evident in the ^1H NMR paramagnetic chemical shift due to acylation; the carbinyl proton at the glycerol C-2 was deshielded by 1.07 ppm in comparison with that of methylregaloside A [6] to appear at δ5.14 (*m*). Thus, the structure of **14** was established as (2*R*)-1-*O*-β-D-glucopyranosyl-2-*O*-*p*-coumaroylglycerol, designated as regaloside H.

The spectral properties of **15** were essentially identical with those of **14**. The EI mass spectrum showed a molecular ion peak at *m/z* 442 which exceeded that of **14** by 42 mass units, and the ^1H and ^{13}C NMR spectra exhibited signals due to an acetyl group [^1H NMR: δ2.04, 3H, *s*; ^{13}C NMR: δ172.6 and 20.7 (Table 1)]. The specific rotation, spectral data and TLC behaviour of the acetyl derivative (**15a**) of **15** agreed fully with those of **14a**. Therefore, **15** must be a monoacetate of **14**. The ^{13}C NMR chemical shifts of the glucose residue in **15** agreed with those in **14**, confirming no substitution at the glucose moiety. In the ^1H NMR spectrum of **15**, the signals of the glycerol H-2 and H_2-3 were shifted downfield to appear at δ5.34 (*m*, H-2), 4.44 (*dd*, *J* = 12.0, 3.5 Hz, H-3a), 4.30 (*dd*, *J* = 12.0, 6.6 Hz, H-3b), respectively. From the discussion above, **15** was 3-*O*-acetyl derivative of **14**, and shown to be (2*S*)-1-*O*-β-D-glucopyranosyl-2-*O*-*p*-coumaroyl-3-*O*-acetylglycerol, named regaloside I.

Lilium tenuifolium

From the chloroform-soluble fraction of the methanolic bulb extract of *L. tenuifolium*, **1**–**4** were isolated, and from the *n*-butanol-soluble fraction, **6**–**8**, **11**, **13**, adenosine (**16**), methyl α-D-mannopyranoside (**17**), methyl β-D-fructofuranoside (**18**), D-galactose (**19**) and two new constituents (**20**, **21**) were isolated.





Compound **20** was obtained as a white amorphous powder, which showed slightly bitter taste. The IR spectrum was consistent with the presence of hydroxyl group(s) (3420 cm^{-1}) and a carbonyl group (1695 cm^{-1}), the latter was also revealed by the UV (λ_{max} 279 nm, ε240) and ^{13}C NMR (δ211.4) spectra. Acetylation of **20** by the usual manner gave a pentaacetate (**20a**) as a white amorphous powder, and the EI mass spectrum showed a fragment ion peak at *m/z* 762 $[M-MeCOOH]^+$. The ^1H NMR spectrum of **20** contained signals for two secondary methyl groups at δ0.84 (*d*, $J=6.5$ Hz) and 0.83 (*d*, $J=6.5$ Hz), and three tertiary methyl groups at δ1.56, 1.34 and 0.73 (each 3H, *s*). The ^{13}C NMR spectrum showed a total of 27 carbons except for the sugar moiety, and the 27 carbons were readily separated to Me × 5, CH_2 × 10, CH × 7 and C × 5 with the help of the DEPT spectrum. The signals at δ83.8, 76.9, 76.8 and 76.7 were apparently due to the carbons bearing oxygen functions. The monosaccharide composing **20** was easily deduced to be β-D-glucose from the ^1H NMR (δ5.04, *d*, $J=7.7$ Hz, anomeric proton) and the ^{13}C NMR (δ102.0, 78.6 × 2, 75.4, 71.9 and 63.0) spectra measured in C_5D_5N. The above results were indicative of the fundamental skeleton of **20** being a cholestane glucoside with two secondary hydroxyl groups, two tertiary hydroxyl groups and a carbonyl group. On enzymatic hydrolysis with β-glucosidase, **20** was cleaved to yield D-glucose and the steroidal genin (**20b**). The molecular formula, $C_{27}H_{46}O_5$ was obtained from the high resolution mass spectrum. The ^{13}C NMR chemical shifts of the D ring and the side chain in **20** and **20b** were closely related to those in ponasterone A [19], whose C-14, C-20 and C-22 positions bear hydroxyl functions. In the ^1H NMR spectrum of **20b**, the signals assignable to the H-22 appeared at δ3.80 (*br d*, $J=9.6$ Hz), on acetylation (**20c**), which was shifted to lower field at δ4.82 (*dd*, $J=10.6$, 2.2 Hz). The base peak at *m/z* 331.2261 ($C_{21}H_{31}O_3$) in the EI mass spectrum of **20b** was assumed to be produced by the results of a bond fission between the C-20 and C-22 and the elimination of H_2O. In the ^{13}C NMR spectrum of **20b**, the signals arising from the C-9 (δ46.7), C-12 (δ33.0) and C-17 (δ50.2) were displaced upfield by the 1,3-diaxial interactions with the α-hydroxyl group at the C-14 position, while the C-13 (δ48.8), C-15 (δ32.4) and C-18 (δ17.8) moved downfield, compared with the signals of (20*R*, 22*R*)-dihydroxy-5α-cholestanol [19]. Thus, the presence of 14α, 20 and 22 hydroxyl groups was evident. The ^1H–^1H COSY spectrum of **20** (CD_3OD) made it possible to assign the signals at δ2.28 (1H, *dd*, $J=12.2$, 2.2 Hz), 2.48 (1H, *dd*, $J=12.7$, 12.7 Hz), 2.09 (1H, *dd*, $J=12.7$, 4.5 Hz) and 2.17 (1H, *ddd*, $J=12.7$, 12.7, 4.5 Hz) to the H-5, H-7 axial, H-7 equatorial and H-8 protons, respectively. Furthermore, the CD spectrum of **20** indicated a negative maximum at 291 nm, which was compatible with the presence of the C-6 carbonyl group [20]. In the ^{13}C NMR spectrum of **20b**, the signal attributed to the C-3 was remarkably shifted to upper field by 6.8 ppm, whereas the signals due to the C-2 and C-4 were shifted to lower field as compared with those of **20**. Therefore, the glucose moiety was linked to the C-3 hydroxyl position on the genin. The configuration of the C-3 hydroxyl group was confirmed to be β as the proton on the C-3 was observed as the broad multiplet signal ($W_{1/2}=28.4$ Hz) in the ^1H NMR spectrum of **20** (C_5D_5N). The ^{13}C NMR chemical shift of the C-19 methyl group in **20** was suggestive of the A/B *trans*-junction [21, 22]. From the above properties, **20** was expected to be 6-oxo-14α,20,22-trihydroxy-5α-cholestanol 3-*O*-glucoside, and it remained to determine the configurations at the C-20 and C-22. The NMR analytical method is available to differentiate the configurations of the C-20 and/or C-22 hydroxyl isomers having the cholestane side chain [23–28]. For example, the ^1H NMR signal of the H-22 methine proton differs by 0.45 ppm between (20*R*,22*R*)-3α,5-cyclo-5α-cholestane-6β,20,22-triol 6-methyl ether and its C-22 epimer [27]. Ponasterone A (**22**) and B (**23**) are 14α, (20*R*), (22*R*)-hydroxy phytoecdysones [29–31]. The ^{13}C NMR chemical shifts of the D ring and side chain of **20** and **20b** were identical to those of ponasterone A (**22**) as shown in Table 2, and the ^1H NMR of the H-21, H-22 and H-26/27 protons of **20** and **20b**, and the corresponding acetyl derivatives (**20a**, **20c**) to those of ponasterone B and its acetate [30] as shown in Table 3. The configurations in question were concluded to be 20*R* and 22*R*. Thus, the structure of **20** was elucidated to be

Table 2. ^{13}C NMR spectral data for compounds **20**, **20b**, **21** and ponasterone A (**22**)

C	**20**	**20b**	**21**	ponasterone A* (**22**)
Aglycone moiety				
1	37.0[a]	37.3[a]	37.0[a]	37.9
2	27.1	31.4	27.2	68.0
3	76.9[b]	70.1	76.9[b]	68.0
4	29.6	32.0	29.6	32.4
5	56.4	56.9	56.4	51.3
6	211.4	211.8	211.4	203.5
7	42.8	42.9	42.8	121.7
8	40.9	41.0	40.9	166.0
9	46.7	46.8	46.7	34.4
10	40.7	40.8	40.7	38.7
11	21.2[c]	21.3	21.2	21.4
12	33.0[d]	33.1	33.0	31.8
13	48.8	48.9	48.8	48.1
14	83.8	83.9	83.9	84.1
15	32.4[d]	32.4	32.4	31.9
16	21.6[c]	21.7	21.7	21.4
17	50.2	50.2	50.2	50.0
18	17.8	17.8	17.8	17.9
19	12.8	13.0	12.8	24.4
20	76.8	76.8	76.8	76.7
21	21.3	21.3	21.3	21.1
22	76.7[b]	76.8	76.7[b]	76.7
23	30.3	30.3	30.2	30.2
24	37.2[a]	37.2[a]	37.2[a]	37.1
25	28.2	28.2	28.2	28.1
26	22.5	22.5	22.5	22.3
27	23.2	23.3	23.2	23.3
Sugar moiety				
1′	102.0	—	99.6	—
2′	75.4	—	73.0	—
3′	78.6	—	72.4	—
4′	71.9	—	69.3	—
5′	78.6	—	76.0	—
6′	63.0	—	63.2	—

Spectra were measured in C_5D_5N.

*Data are cited from ref. [19].

[a–d]Signals may be interchangeable in each vertical column.

Table 3. Comparison of the ^{1}H NMR spectral data for compounds **20**, **20a**, **20b**, **20c**, ponasterone B (**23**) and ponasterone B acetate

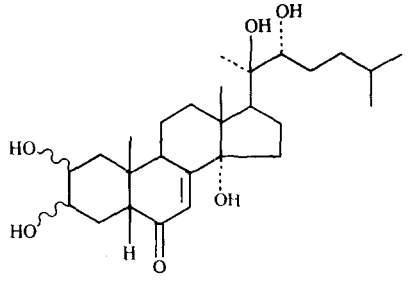
	H-21	H-22	H-26/27
20	1.56	3.79	0.84/0.83
20b	1.58	3.80	0.83/0.82
ponasterone B (**23**)	1.54	—	0.82
20a	1.23	4.82	0.88/0.87
20c	1.23	4.82	0.88/0.87
ponasterone B acetate	1.25	4.82	0.88

Chemical shifts were expressed in ppm relative to int. standard, TMS. Spectra of **20**, **20b** and ponasterone B were run in C_5D_5N, and those of **20a**, **20c** and ponasterone B acetate in $CDCl_3$. Data of ponasterone B and its acetate are cited from ref. [30].

(20*R*, 22*R*)-3β, 20, 22-tetrahydroxy-5α-cholestan-6-one 3-*O*-β-D-glucopyranoside, designated as tenuifolioside A and the steroidal genin (**20b**) as tenuifoliol.

Compound **21** was obtained as a white amorphous powder with the elementary composition $C_{33}H_{56}O_{10}$ from the SI mass spectrum *m/z* 635 $[M+Na]^+$. On acetylation, it gave the corresponding pentaacetate (**21a**) as in **20**. The steroidal skeleton of **21** was confirmed to be the same as that of **20** because of the excellent agreement of the spectral data of the aglycone moieties between **20** and **21**. In the ^{1}H NMR spectrum of **21** and **21a**, the multiplicity and spin–spin coupling constants of the sugar moiety indicated that the hexose constituting **21** was the C-3 epimer of β-D-glucose, that is, β-D-allose. Acid hydrolysis of **20** in refluxing 10% hydrochloric acid yielded the sugar moiety, which was detected by TLC and unambiguously identified as D-allose. The structure of **21** was formulated as (20*R*,22*R*)-3β,14α,20,22-tetrahydroxy-5α-cholestan-6-one 3-*O*-β-D-allopyranoside, designated as tenuifolioside B.

Results of the chemical analysis of the bulbs of *L. auratum* var. *platyphyllum* are interesting in regard to chemotaxonomy. *Lilium auratum* var. *auratum* is endemic to the mainland of Japan, whereas the habitat of *L. auratum* var. *platyphyllum* is limited to Izu Islands and Izu Peninsula, near Tokyo. It is considered that the latter evolved from the former and both the plants are very similar except for their size, but they differ in distribution of regalosides [8]. Regaloside H and I are the first examples bearing the phenylpropanoid moiety at the C-2 position on the glycerol. *Lilium tenuifolium* is widely distributed in northern China and has been used for medicinal material [1, 2]. The occurrence of **20** and **21** seems to be specific to *L. tenuifolium* of the *Lilium* plants.



22 2β, 3β
23 2α, 3α

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR (400 MHz), ^{1}H–^{1}H 2D COSY (500 MHz) and ^{13}C NMR (100.6 MHz): TMS as int. standard. Assignments of the NMR spectra were achieved on the basis of double resonance experiments, ^{1}H–^{1}H COSY and ^{13}C DEPT spectra, and by the correlation with previously reported compounds. Prep. TLC: precoated Kieselgel 60 F_{254} (0.5 mm thick, Merck). Prep. HPLC: CIG column system (Kusano Scientific Co., Tokyo) with Pre-packed Column, 20ϕ × 100 mmL (ODS, 20 μm).

Extraction and isolation. The dormant fresh bulbs of *L. auratum* var. *platyphyllum* (5.7 kg), purchased from Sakata-shubyo Co. Ltd, were cut into pieces and extracted with MeOH under reflux. The extract was concd to almost dryness under red. pres., and the crude product, after dilution with H_2O, was extracted with $CHCl_3$, and then with *n*-BuOH. The $CHCl_3$ soln was repeatedly subjected to silica gel CC with *n*-hexane–Me_2CO, $CHCl_3$–EtOAc, $CHCl_3$–Me_2CO and $CHCl_3$–MeOH solvent systems, and Sephadex LH-20 CC with $CHCl_3$ and MeOH as the eluents to afford compounds **1** (36.2 mg), **2** (63.2 mg), **3** (33.2 mg), **4** (19.0 mg), and **5** (26.3 mg). The *n*-BuOH fraction was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH, $CHCl_3$–MeOH–H_2O, EtOAc–Me_2CO, EtOAc–MeOH, EtOAc–MeOH–H_2O and Et_2O–MeOH solvent systems, and Sephadex LH-20 with MeOH to yield **6** (102 mg), **7** (184 mg), **8** (657 mg), **9** (135 mg), **10** (51.6 mg), **11** (464 mg), **12** (26.7 mg), **13** (111 mg), **14** (36.8 mg) and **15** (10.1 mg). Purification of **11** and **13** was carried out by prep. TLC with EtOAc–2-butanone–MeOH–H_2O (10:10:1:1), and purification of **14** and **15** by prep. HPLC with H_2O–MeOH (13:7). The dormant fresh bulbs of *L. tenuifolium* (5.1 kg), purchased from Heiwaen Co. were treated as in the case of those of *L. auratum* var. *platyphyllum* to provide **1** (35.2 mg), **2** (28.0 mg), **3** (5.1 mg), **4** (3.8 mg), **6** (130 mg), **7** (421 mg), **8** (49.6 mg), **11** (39.6 mg), **13** (6.7 mg), **16** (96.0 mg), **17** (124 mg), **18** (1.56 g), **19** (94.2 mg), **20** (113 mg) and **21** (117 mg).

Regaloside H (**14**). A pale-yellow amorphous powder, $[\alpha]_D^{28}$ −32.0° (MeOH; *c* 0.58). UV λ_{max}^{MeOH} nm (log ε): 227 (4.17), 300 sh (4.36), 313 (4.42). UV $\lambda_{max}^{MeOH+NaOMe}$ nm: 310, 361. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 2900 (CH), 1680 (C=O), 1625 (CH=CH), 1600, 1580, 1510 (aromatic ring), 1440, 1255, 1200, 1160, 1070, 1020, 825. EIMS *m/z* (rel. int.): 400 $[M]^+$ (1.2), 249 (14), 238 (12), 221 (12), 164 (30), 163 (67), 147 (100), 145 (82), 127 (32), 121 (77), 103 (99). ^{1}H NMR (CD_3OD): δ 7.67 (1H, *d*, *J* = 15.9 Hz, H-7'), 7.46 (2H, *d*, *J* = 8.7 Hz, H-2', -6'), 6.81 (2H, *d*, *J* = 8.7 Hz, H-3', -5'), 6.35 (1H, *d*, *J* = 15.9 Hz, H-8'), 5.14 (1H, *m*, H-2), 4.33 (1H, *d*, *J* = 7.8 Hz, H-1''), 4.05 (1H, *dd*, *J* = 10.9, 5.5 Hz, H-1a), 3.87 (1H, *dd*, *J* = 11.9, 1.4 Hz, H-6''a), 3.83 (1H, *dd*, *J* = 10.9, 5.3 Hz, H-1b), 3.82 (1H, *dd*, *J* = 12.0, 4.5 Hz, H-3a), 3.78 (1H, *dd*, *J* = 12.0, 5.4 Hz, H-3b), 3.67 (1H, *dd*, *J* = 11.9, 5.3 Hz, H-6''b), 3.40–3.28 (overlapping with solvent signal, H-3'', -4'', -5''), 3.21 (1H, *dd*, *J* = 9.0, 7.8 Hz, H-2'').

Acetylation of compound **14**. The hexaacetate (**14a**) (7.3 mg) of **14** was prepared by allowing a pyridine soln of **14** (7.4 mg) to stand at room temp. overnight in the presence of excess Ac_2O. Extraction with $CHCl_3$, after addition of H_2O, gave a solid residue which was chromatographed on silica gel with *n*-hexane–Me_2CO (2:1). Compound **14a** was obtained as a white amorphous powder, $[\alpha]_D^{28}$ −7.1° ($CHCl_3$; *c* 0.31). IR ν_{max}^{KBr} cm^{-1}: 2960 (CH), 1755 (C=O), 1635 (CH=CH), 1600, 1510 (aromatic ring), 1370, 1220, 1160, 1035, 985, 905, 835. EIMS *m/z* (rel. int.):

652 $[M]^+$ (weak), 610 (4.7), 331 (17), 305 (46), 263 (28), 189 (28), 169 (64), 147 (100), 127 (10), 109 (37). ^{1}H NMR ($CDCl_3$): δ7.69 (1H, *d*, J = 16.0 Hz, H-7′), 7.56 (2H, *d*, J = 8.6 Hz, H-2′, -6′), 7.14 (2H, *d*, J = 8.6 Hz, H-3′, -5′), 6.38 (1H, *d*, J = 16.0 Hz, H-8′), 5.31 (1H, *m*, H-2), 5.21 (1H, *dd*, J = 9.5, 9.5 Hz, H-3″), 5.08 (1H, *dd*, J = 9.5, 9.5 Hz, H-4″), 5.00 (1H, *dd*, J = 9.5, 7.9 Hz, H-2″), 4.57 (1H, *d*, J = 7.9 Hz, H-1″), 4.34 (1H, *dd*, J = 12.0, 3.7 Hz, H-3a), 4.26 (1H, *dd*, J = 12.4, 4.8 Hz, H-6″a), 4.25 (1H, *dd*, J = 12.0, 6.4 Hz, H-3b), 4.14 (1H, *dd*, J = 12.4, 2.3 Hz, H-6″b), 4.02 (1H, *dd*, J = 11.1, 4.6 Hz, H-1a), 3.79 (1H, *dd*, J = 11.1, 5.3 Hz, H-1b), 3.70 (1H, *ddd*, J = 9.5, 4.8, 2.3 Hz, H-5″), 2.32 (3H, *s*, arom. Ac), 2.09, 2.07, 2.04, 2.02, 2.00 (each 3H, *s*, Ac × 5).

Basic hydrolysis followed by acetylation of compound **14**. Hydrolysis of **14** (10.7 mg) with 3% NaOMe in MeOH was carried out at room temp. for 2 hr. After addition of MeOH, the reaction soln was passed through a cation exchange resin (Amberlite IR–120B). Methyl ferulate was detected in the reaction mixture by TLC, R_f 0.62 ($CHCl_3$–MeOH; 15:1). The mixture was subsequently acetylated with Ac_2O in pyridine, and the crude product was purified by silica gel CC with *n*-hexane–Me_2CO (2:1) to give lilioside C hexaacetate (10.5 mg) [5, 18].

Regaloside I (**15**). A pale-yellow amorphous powder, $[\alpha]_D^{28}$ − 11.1° (MeOH; *c* 0.51). UV λ_{max}^{MeOH} nm (logε): 226 (4.08), 300 sh (4.29), 312 (4.34). UV $\lambda_{max}^{MeOH + NaOMe}$ nm: 310, 362. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2910 (CH), 1705 (C=O), 1620 (CH=CH), 1595, 1580, 1505 (aromatic ring), 1440, 1360, 1320, 1250, 1160, 1065, 1030, 825. EIMS *m/z* (rel. int.): 442 $[M]^+$ (2), 351 (0.9), 308 (6.6), 280 (33), 263 (65), 249 (24), 247 (22), 238 (12), 221 (17), 205 (41), 187 (37), 164 (60), 163 (37), 147 (100), 145 (100), 127 (53), 117 (99), 103 (100). ^{1}H NMR (CD_3OD): δ7.65 (1H, *d*, J = 15.9 Hz, H-7′), 7.46 (2H, *d*, J = 8.6 Hz, H-2′, -6′), 6.81 (2H, *d*, J = 8.6 Hz, H-3′, -5′), 6.33 (1H, *d*, J = 15.8 Hz, H-8′), 5.34 (1H, *m*, H-2), 4.44 (1H, *dd*, J = 12.0, 3.5 Hz, H-3a), 4.31 (1H, *d*, J = 7.8 Hz, H-1″), 4.30 (1H, *dd*, J = 12.0, 6.6 Hz, H-3b), 4.06 (1H, *dd*, J = 11.1, 5.3 Hz, H-1a), 3.87 (1H, *dd*, J = 11.9, 1.9 Hz, H-6″a), 3.81 (1H, *dd*, J = 11.1, 5.4 Hz, H-1b), 3.67 (1H, *dd*, J = 11.9, 5.4 Hz, H-6″b), 3.38–3.27 (overlapping with solvent signal, H-3″, -4″, -5″), 3.20 (1H, *dd*, J = 9.0, 7.8 Hz, H-2″), 2.04 (3H, *s*, Ac).

Acetylation of compound **15**. Compound **15** (4.8 mg) was acetylated with Ac_2O in pyridine and the crude acetate was subjected to silica gel CC with *n*-hexane–Me_2CO (2:1) to give a peracetate (**15a**) (6.0 mg). The specific rotation and the spectral data (IR and ^{1}H NMR spectra) coincided with those of **14a**.

Tenuifolioside A (**20**). A white amorphous powder, $[\alpha]_D^{28}$ − 9.8° (MeOH; *c* 0.54). UV λ_{max}^{MeOH} nm (ε): 230 sh (714), 279 (240), 316 sh (159). CD (MeOH; *c* 0.000882) nm (θ): 291 (−4762). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2950, 2870 (CH), 1695 (C=O), 1465, 1455, 1380, 1360, 1250, 1165, 1075, 1030, 955, 905, 890. SIMS *m/z*: 433 [aglycone − OH]$^+$, 415 [aglycone − OH − H_2O]$^+$, 397 [aglycone − OH − 2H_2O]$^+$. ^{1}H NMR (C_5D_5N): δ5.04 (1H, *d*, J = 7.7 Hz, H-1′), 4.61 (1H, *dd*, J = 11.7, 2.2 Hz, H-6′a), 4.39 (1H, *dd*, J = 11.7, 5.6 Hz, H-6′b), 4.28 (1H, *dd*, J = 8.7, 8.7 Hz, H-3′), 4.22 (1H, *dd*, J = 8.7, 8.7 Hz, H-4′), 4.04 (1H, *dd*, J = 8.7, 7.7 Hz, H-2′), 4.00 (1H, *ddd*, J = 8.7, 5.6, 2.2 Hz, H-5′), 3.94 (1H, *br m*, $W_{1/2}$ = 28.4 Hz, H-3), 3.79 (1H, *br d*, J = 10.3 Hz, H-22), 2.88 (1H, *dd*, J = 12.7, 12.7 Hz, H-7 axial), 2.84 (1H, *t*, J = 9.5 Hz, H-17), 2.45 (1H, *dd*, J = 12.7, 4.3 Hz, H-7 equatorial), 2.29 (1H, *ddd*, J = 12.7, 12.7, 4.2 Hz, H-8), 2.13 (1H, *ddd*, J = 12.7, 12.7, 4.2 Hz, H-9), 1.56 (3H, *s*, H-21), 1.34 (3H, *s*, H-18), 0.84 (3H, *d*, J = 6.5 Hz, H-26 or -27), 0.83 (3H, *d*, J = 6.5 Hz, H-26 or -27), 0.73 (3H, *s*, H-19). ^{1}H NMR (CD_3OD): δ4.39 (1H, *d*, J = 7.8 Hz, H-1′), 3.85 (1H, *dd*, J = 12.3, 1.7 Hz, H-6′a), 3.70 (1H, *br m*, $W_{1/2}$ = 22.9 Hz, H-3), 3.64 (1H, *dd*, J = 12.3, 5.4 Hz, H-6′b), 3.37–3.21 (overlapping with solvent signal, H-22, -3′, -4′, -5′), 3.14 (1H, *dd*, J = 9.0, 7.8 Hz, H-2′), 2.48 (1H, *dd*, J = 12.7, 12.7 Hz, H-7 axial), 2.31 (1H, *t*, J = 9.3 Hz, H-17), 2.28 (1H, *dd*, J = 12.2, 2.2 Hz, H-5), 2.17 (1H, *ddd*, J = 12.7, 12.7, 4.5 Hz, H-8), 2.09 (1H, *dd*, J = 12.7, 4.5 Hz, H-7 equatorial), 1.15 (3H, *s*, H-21), 1.02 (3H, *s*, H-18), 0.91 (3H, *d*, J = 6.5 Hz, H-26 or -27), 0.90 (3H, *d*, J = 6.5 Hz, H-26 or -27), 0.76 (3H, *s*, H-19).

Acetylation of compound **20**. A mixture of **20** (12.6 mg), Ac_2O and pyridine was left standing overnight. The crude acetate was chromatographed on a silica gel column using $CHCl_3$–EtOAc (3:1) and Et_2O to give a white amorphous powder (**21**) (9.4 mg). IR ν_{max}^{KBr} cm^{-1}: 2950, 2870 (CH), 1750, 1705 (C=O), 1460, 1365, 1220, 1160, 1030, 955, 900. EIMS *m/z* (rel. int.): 762 [M − AcOH]$^+$ (1.3), 744 [M − AcOH − H_2O]$^+$ (3.5), 726 [M − AcOH − 2H_2O]$^+$ (4.2), 659 (2), 616 (2.7), 457 (2.3), 415 (19), 397 (78), 379 (20), 331 (50), 299 (13), 269 (18), 242 (12), 205 (20), 169 (100), 157 (21), 150 (23), 145 (18), 109 (45). ^{1}H NMR ($CDCl_3$): δ5.18 (1H, *dd*, J = 9.5, 9.5 Hz, H-3′), 5.08 (1H, *dd*, J = 9.5, 9.5 Hz, H-4′), 4.96 (1H, *dd*, J = 9.5, 7.9 Hz, H-2′), 4.82 (1H, *dd*, J = 10.4, 2.2 Hz, H-22), 4.61 (1H, *d*, J = 7.9 Hz, H-1′), 4.25 (1H, *dd*, J = 12.3, 4.7 Hz, H-6′a), 4.12 (1H, *dd*, J = 12.3, 2.5 Hz, H-6′b), 3.66 (1H, *ddd*, J = 9.5, 4.7, 2.5 Hz, H-5′), 3.56 (1H, *br m*, $W_{1/2}$ = 23.6 Hz, H-3), 2.11, 2.08, 2.07, 2.02, 2.00 (each 3H, *s*, Ac × 5), 1.23 (3H, *s*, H-21), 1.00 (3H, *s*, H-18), 0.88 (3H, *d*, J = 6.6 Hz, H-26 or -27), 0.87 (3H, *d*, J = 6.6 Hz, H-26 or -27), 0.75 (3H, *s*, H-19).

Enzymatic hydrolysis of **20**. A mixture of **20** (50.0 mg) and β-glucosidase (10.0 mg) was incubated at 37° for 5 hr in HOAc–NaOAc buffer (pH 5.0). The reaction mixture was diluted with H_2O and then extracted with EtOAc, which was subjected to silica gel CC using $CHCl_3$–Me_2CO (4:1) and $CHCl_3$–MeOH (19:1), and Sephadex LH-20 CC using MeOH to elute the aglycone (**20b**) (27.9 mg). Colourless needles recrystallized from MeOH, mp 227.5–232.5°, $[\alpha]_D^{26}$ + 16.8° ($CHCl_3$–MeOH, 2:1; *c* 0.25). IR ν_{max}^{KBr} cm^{-1}: 3430, 3320 (OH), 2935, 2850 (CH), 1690 (C=O), 1455, 1395, 1350, 1320, 1295, 1275, 1250, 1235, 1200, 1155, 1105, 1080, 1055, 1035, 990, 960, 950, 920, 905, 885, 795. EIMS *m/z* (rel. int.): 432 [M − H_2O]$^+$ (weak), 414.3160 [M − 2H_2O]$^+$ (5.5), calcd for $C_{27}H_{42}O_3$: 414.3136, 396 (4.1), 331.2261 [M − $C_6H_{13}O$ − H_2O]$^+$ (100), calcd for $C_{21}H_{31}O_3$: 331.2274, 313 (51), 295 (18), 286 (57), 272 (14), 253 (9), 201 (10), 175 (19), 159 (18), 147 (28), 145 (28), 133 (34), 105 (42). ^{1}H NMR (C_5D_5N): δ3.81 (1H, *br m*, H-3), 3.80 (1H, *br d*, J = 9.6 Hz, H-22), 2.92 (1H, *dd*, J = 12.6, 12.6 Hz, H-7 axial), 2.86 (1H, *t*, J = 9.4 Hz, H-17), 2.48 (1H, *dd*, J = 12.6, 4.4 Hz, H-7 equatorial), 1.58 (3H, *s*, H-21), 1.38 (3H, *s*, H-18), 0.86 (3H, *s*, H-19), 0.83 (3H, *d*, J = 6.5 Hz, H-26 or -27), 0.82 (3H, *d*, J = 6.5 Hz, H-26 or -27). The H_2O residue was purified with silica gel CC with $CHCl_3$–MeOH–H_2O (40:20:1) to yield D-glucose; TLC, R_f 0.25 (*n*-BuOH–Me_2CO–H_2O, 4:5:1), $[\alpha]_D^{26}$ + 58.4° (H_2O; *c* 0.49).

Acetylation of compound **20b**. Compound **20b** (5.0 mg) was acetylated with Ac_2O in pyridine. Purification of the crude product was carried out by silica gel CC with $CHCl_3$–EtOAc (4:1) to afford the corresponding diacetate (**20c**) (3.5 mg) as a white amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3510 (OH), 2940, 2850 (CH), 1725, 1680 (C=O), 1460, 1360, 1250, 1225, 1155, 1030, 955, 940, 900, 880, 795. ^{1}H NMR ($CDCl_3$): δ4.82 (1H, *dd*, J = 10.6, 2.2 Hz, H-22), 4.67 (1H, *br m*, $W_{1/2}$ = 23.1 Hz, H-3), 2.11, 2.03 (each 3H, *s*, Ac × 2), 1.23 (3H, *s*, H-21), 1.00 (3H, *s*, H-18), 0.88 (3H, *d*, J = 6.6 Hz, H-26 or − 27), 0.87 (3H, *d*, J = 6.6 Hz, H-26 or -27), 0.78 (3H, *s*, H-19).

Tenuifolioside B (**21**). A white amorphous powder, $[\alpha]_D^{26}$ − 19.0° (MeOH; *c* 0.20). UV λ_{max}^{EtOH} nm (ε): 230 sh (589), 281 (249), 320 sh (147). CD (EtOH; *c* 0.000654) nm (θ): 290 (−5199). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2945, 2870 (CH), 1695 (C=O), 1460, 1380, 1360, 1245, 1075, 1020, 955, 900, 880. SIMS *m/z*: 635 [M + Na]$^+$. ^{1}H NMR (C_5D_5N): δ5.49 (1H, *d*, J = 7.8 Hz, H-1′), 4.76 (1H, *dd*, J = 2.8, 2.8 Hz, H-3′), 4.57 (1H, *dd*, J = 11.6, 2.1 Hz, H-6′a), 4.51 (1H, *ddd*, J = 9.6, 5.3, 2.1 Hz, H-5′), 4.39 (1H, *dd*, J = 11.6, 5.3 Hz, H-6′b), 4.22 (1H, *dd*, J = 9.6, 2.8 Hz, H-4′), 3.98 (1H, *dd*, J = 7.8, 2.8 Hz, H-2′), 3.89 (1H, *br m*, $W_{1/2}$ = 24.5 Hz, H-3), 3.79

(1H, *br d*, $J = 10.2$ Hz, H-22), 2.87 (1H, *dd*, $J = 12.6$, 12.6 Hz, H-7 axial), 2.85 (1H, *t*, $J = 9.1$ Hz, H-17), 2.44 (1H, *dd*, $J = 12.6$, 4.5 Hz, H-7 equatorial), 2.28 (1H, *ddd*, $J = 12.6$, 12.6, 4.2 Hz, H-8), 2.12 (1H, *ddd*, $J = 12.8$, 12.8, 4.2 Hz, H-9), 1.57 (3H, *s*, H-21), 1.34 (1H, *s*, H-18), 0.84 (1H, *d*, $J = 6.4$ Hz, H-26 or -27), 0.83 (3H, *d*, $J = 6.5$ Hz, H-26 or -27), 0.72 (3H, *s*, H-19). ^{1}H NMR (CD_3OD): δ4.76 (1H, *d*, $J = 8.0$ Hz, H-1'), 4.04 (1H, *dd*, $J = 3.0$, 3.0 Hz, H-3'), 3.82 (1H, *br d*, $J = 9.6$ Hz, H-6'a), 3.73–3.58 (3H, overlapping, H-3, -5', –6'b), 3.46 (1H, *dd*, $J = 9.5$, 3.0 Hz, H-4'), 3.31 (overlapping with solvent signal, H-22), 3.26 (1H, *dd*, $J = 8.0$, 3.0 Hz, H-2'), 2.49 (1H, *dd*, $J = 12.6$, 12.6 Hz, H-7 axial), 2.31 (1H, *t*, $J = 9.4$ Hz, H-17), 2.28 (1H, *dd*, $J = 12.4$, 2.4 Hz, H-5), 2.17 (1H, *ddd*, $J = 12.6$, 12.6, 4.4 Hz, H-8), 2.08 (1H, *dd*, $J = 12.6$, 4.4 Hz, H-7 equatorial), 1.15 (3H, *s*, H-21), 1.02 (3H, *s*, H-18), 0.91 (3H, *d*, $J = 6.5$ Hz, H-26 or -27), 0.90 (3H, *d*, $J = 6.5$ Hz, H-26 or -27), 0.76 (3H, *s*, H-19).

Acetylation of compound **21**. A soln of **21** (30.0 mg) in Ac_2O and pyridine was allowed to stand at room temp. overnight. The crude acetate was chromatographed on silica gel with $CHCl_3$–EtOAc (3:1) to provide a white amorphous powder (**21a**) (26.9 mg). IR ν_{max}^{KBr} cm^{-1}: 2940 (CH), 1745, 1700 (C=O), 1365, 1250, 1220, 1160, 1080, 1030, 955, 940, 900, 795. EIMS *m/z* (rel. int.): 762 $[M-AcOH]^+$ (weak), 744 $[M-AcOH-H_2O]$ (3.7), 726 $[M-AcOH-2H_2O]^+$ (6.5), 661 (2.7), 616 (1.3), 577 (0.9), 503 (3.7), 429 (8), 415 (11), 397 (27), 379 (22), 355 (13), 331 (100), 313 (27), 281 (13), 221 (20), 187 (13), 169 (47), 147 (32), 109 (29). ^{1}H NMR ($CDCl_3$): δ5.64 (1H, *d*, $J = 2.9$, 2.9 Hz, H-3'), 4.96 (1H, *dd*, $J = 10.2$, 2.9 Hz, H-4'), 4.91 (1H, *d*, $J = 8.2$ Hz, H-1'), 4.84 (1H, *dd*, $J = 8.2$, 2.9 Hz, H-2'), 4.82 (1H, *d*, $J = 9.7$, 2.1 Hz, H-22), 4.21 (1H, *dd*, $J = 12.2$, 4.2 Hz, H-6'a), 4.17 (1H, *dd*, $J = 12.2$, 2.8 Hz, H-6'b), 4.02 (1H, *ddd*, $J = 10.2$, 4.2, 2.8 Hz, H-5'), 3.61 (1H, *br m*, $W_{1/2} = 24.8$ Hz, H-3), 2.16, 2.11, 2.08, 2.04, 1.99 (each 3H, *s*, Ac × 5), 1.23 (3H, *s*, H-21), 1.01 (3H, *s*, H-18), 0.88 (3H, *d*, $J = 6.6$ Hz, H-26 or -27), 0.87 (3H, *d*, $J = 6.6$ Hz, H-26 or -27), 0.77 (3H, *s*, H-19).

Acid hydrolysis of compound **21**. A sample of **21** (40.0 mg) in 10% HCl (H_2O–dioxane, 2:1) was heated under reflux for 3 hr. The reaction mixture was neutralized with 10% NaOH, and then chromatographed on a silica gel column using $CHCl_3$–MeOH (2:1) to yield D-allose (12.7 mg). TLC, R_f 0.33 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). $[\alpha]_D^{25}$ + 14.8° (H_2O; *c* 0.64). The aglycone moiety seemed to be decomposed through the hydrolysis.

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