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REGALOSIDE A AND B, ACYLATED GLYCEROL GLUCOSIDES FROM *LILIUM REGALE*

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Key Word Index—*Lilium regale*; Liliaceae; acylated glycerol glucosides; *p*-coumaric acid esters; regaloside A; regaloside B; bitter principles; stereochemistry.

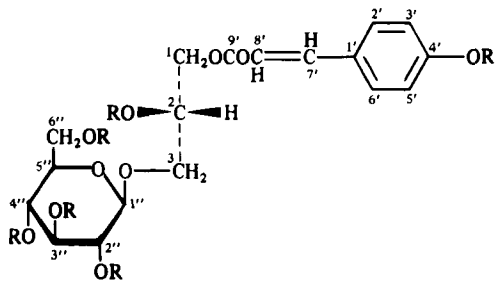
Abstract—Novel acylated glycerol glucosides, regaloside A and B, both bitter to the taste, have been isolated from the fresh bulbs of *Lilium regale*. Their structures have been shown by the spectral and chemical evidence to be (2*S*)-1-*O*-*p*-coumaroyl-3-*O*-β-D-glucopyranosylglycerol and (2*S*)-1-*O*-*p*-coumaroyl-2-*O*-β-D-glucopyranosyl-3-*O*-acetylglycerol, respectively.

INTRODUCTION

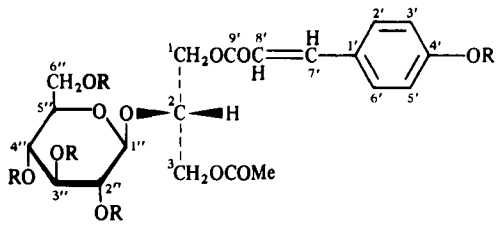
The dried bulbs of the genus *Lilium* have been used as a crude drug in traditional Chinese medical preparations. However, no effective constituents have been identified.

Our previous works on the chemical constituents of the genus *Lilium* have led to the isolation and the structure elucidation of seven bitter phenylpropanoid glycosides, 3,6′-diferuloylsucrose and its acetyl derivatives from *Lilium speciosum* var. *rubrum* [1]; an antitumour alkaloid, jatropham and its glucoside from *Lilium hansonii* [2] and new phenolic glycerides, 1,2-diacylated glycerols from *Lilium auratum* [3]. As a part of our chemical studies on the bulbs of *Lilium* plants, an examination have been made of *Lilium regale* Wilson, for which no report has appeared on the chemical principles except for the amino acid related compounds [4], and hydrocarbons [5].

From the methanol extract of this plant, two novel bitter glucosides, for which we propose the names regaloside A (**1**) and regaloside B (**2**), were isolated. The structures and the absolute configurations of **1** and **2** were established on the basis of spectroscopic analysis and chemical degradation, and by the chemical correlation with a previously described compound [6].



1 R = H
1a R = Ac



2 R = H
2a R = Ac

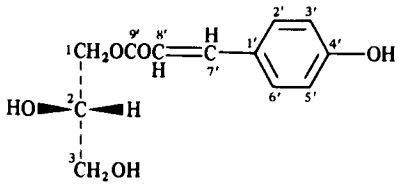
RESULTS AND DISCUSSION

The methanol extract of fresh bulbs of *L. regale* was separated into a chloroform-soluble portion and *n*-butanol-soluble portion. The latter was further fractionated through the combined use of repeated silica gel, Sephadex LH-20 column chromatography and then preparative TLC as described in Experimental, resulting in the isolation of **1** and **2**.

Regaloside A (**1**), $C_{18}H_{24}O_{10}$, was obtained as a pale-yellow amorphous powder, $[\alpha]_D$ −15.2° (MeOH), and gave a positive coloration with benzidine reagent. It showed absorption bands of hydroxy groups (3300 cm^{-1}), a carbonyl group of α,β-unsaturated ester (1700 cm^{-1}), an alkene conjugated with an aromatic ring (1640 cm^{-1}) and an aromatic ring (1610, 1510 cm^{-1}) in the IR spectrum. The electron impact mass spectrum (EIMS) of **1** showed a molecular ion peak at *m/z* 400 and the highest fragment ion peak at *m/z* 147, assignable to a *p*-coumaroyl moiety. Furthermore, in the ^1H NMR spectrum, the appearance of two pairs of doublet signals due to

trans-alkene protons (δ7.65 and 6.32, each *d*, $J = 16.0$ Hz) and *p*-disubstituted aromatic protons (7.43 and 6.81, each 2H, *d*, $J = 8.0$ Hz), proved **1** to possess a *p*-coumaroyl unit in the molecule. Acetylation of **1** with acetic anhydride–pyridine afforded the hexaacetate (**1a**) as colourless needles recrystallized from ethanol, mp 137–139°. The EIMS of **1a** gave a molecular ion peak at *m/z* 652 and a fragment ion peak at *m/z* 331, corresponding to the tetraacetylglucose oxonium ion. The ^{1}H NMR spectrum of **1a** exhibited the presence of five aliphatic acetate groups and an aromatic acetate group for the *p*-coumaric acid residue. Enzymatic hydrolysis of **1** with β-glucosidase yielded D-glucose and colourless plates, identical to 1-*O*-*p*-coumaroylglycerol (**3**) [3, 7] according to the IR and ^{1}H NMR spectra. On methanolysis with 3% sodium methoxide, **1** gave methyl *p*-coumarate and a hydrolysate, glycerol glucoside (**4**), which subsequently decomposed on treatment with 10% hydrochloric acid to afford D-glucose and glycerol. On the basis of these findings, **1** might be the 1-*O*-*p*-coumaroyl ester of glycerol glucoside. The exact position of the glucose moiety was confirmed by a periodate oxidation study of **4**, in which **4** consumed *ca* three mol of oxidant with the formation of *ca* one mol each of formic acid and formaldehyde. Therefore, the glucose residue was linked to a primary hydroxy group of glycerol; the anomeric carbon appeared at δ104.7 in the ^{13}C NMR spectrum of **1**, showing the glucose linkage to be the β-orientation. In order to determine the absolute configuration of C-2 of the glycerol, **4** was converted to the corresponding hexaacetate (**4a**) which was shown to be lilioside C hexaacetate [6] by direct comparison with an authentic sample (TLC, ^{1}H NMR spectrum and the mmp). Thus, the structure of **1** was established as (2*S*)-1-*O*-*p*-coumaroyl-3-*O*-β-D-glucopyranosylglycerol. The proposed structure of **1** was supported by the results of the ^{13}C NMR spectrum (Table 1).

Regaloside B (**2**) was obtained as a pale-yellow amorphous powder, $[\alpha]_D$ −21.2° (MeOH), with a molecular formula $C_{20}H_{26}O_{11}$. It was positive to the benzidine test. The spectral data of **1** and **2** were simular and suggestive of glucoside structure of the same type. In the EIMS of **2**, the molecular ion peak at *m/z* 442 exceeded that of **1** by 42 mass units. The IR spectrum of **2** exhibited the presence of an ester carbonyl group (1720 cm^{-1}), and the ^{1}H NMR spectrum showed a signal arising from an aliphatic acetoxyl group at δ2.01 (3H, *s*). These data indicated the presence of an acetoxyl group attached to *p*-coumaroyl glycerol glucoside. On acetylation of **2** with acetic anhydride–pyridine, the corresponding pentaacetate (**2a**) was obtained as colourless needles recrystallized from ethanol, mp 118–120°. The EIMS of **2a** showed a molecular ion peak at *m/z* 652 and a fragment ion peak at *m/z* 331 as for **1a**. However, the IR and ^{1}H NMR spectra of **2a** were not in agreement with those of **1a**. Enzymatic hydrolysis of **2** with β-glucosidase yielded D-glucose and a pale-yellow viscous syrup (**5**). The ^{1}H NMR spectrum of **5** exhibited signals due to *p*-coumaroyl protons, 1,3-diacylated glycerol protons and aliphatic acetoxyl group protons. The structure of **5** was assigned as 1-*O*-*p*-coumaroyl-3-*O*-acetylglycerol. When **2** was submitted to alkaline methanolysis with 3% sodium methoxide, **2** was hydrolysed to yield methyl *p*-coumarate and glycerol glucoside (**6**), which gave D-glucose and glycerol by the subsequent hydrolysis with 10% hydrochloric acid. In a periodate oxidation study, **6** consumed *ca* two mol of oxidant to produce *ca* one mol of formic acid. No formaldehyde was detected in the reaction mixture. These data were consistent only with 2-*O*-glucopyranosylglycerol. The mode of glucosidic linkage was determined to be the β-form based on the anomeric carbon signal (δ104.4) in the ^{13}C NMR spectrum of **2**. Acetylation of **5** afforded the corresponding diacetate (**5a**), having a specific rotation agreeing with that of the triacetate of **3**. Thus, the absolute configuration of



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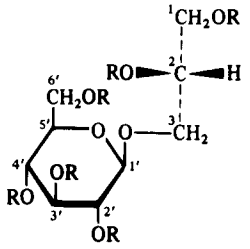
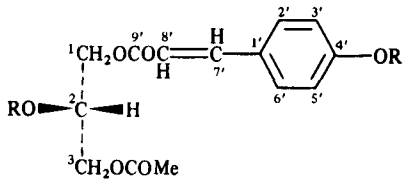


4 R = H
4a R = Ac

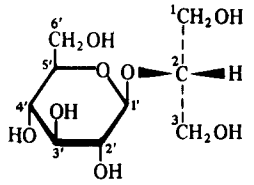
Table 1. ^{13}C NMR spectral data (100.6 MHz) for compounds **1** and **2**

C	1	2
1	66.7	65.1
2	69.7	76.1
3	72.0	64.4
1′	127.2	127.1
2′	131.2	131.3
3′	116.9	116.9
4′	161.3	161.3
5′	116.9	116.9
6′	131.2	131.3
7′	146.8	147.1
8′	115.0	114.8
9′	169.2	168.9
1″	104.7	104.4
2″	75.1	75.0
3″	78.0*	78.0†
4″	71.6	71.5
5″	77.9*	77.9†
6″	62.7	62.8
Ac		172.7
		20.8

Solvent CD_3OD.
*,†Assignments may be interchangeable within each column.



5 R = H
5a R = Ac



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C-2 of the glycerol of **2** was deduced to be *S*. From the arguments presented above, **2** was concluded to be (2*S*)-1-*O*-*p*-coumaroyl-2-*O*-*β*-D-glucopyranosyl-3-*O*-acetylglycerol. The ^{13}C NMR spectrum of **2** was in good agreement with the proposed structure (Table 1).

Some glycerol glycosides have been isolated from red algae and their sugar moieties found to consist of only galactose and/or mannose [8–15]. Recently, new glycerol glucosides, lilioside A–E have been reported as the characteristic constituents of the genus *Lilium* [6, 16, 17]. Regaloside A and B are *p*-coumaroyl esters of glycerol glucoside and new types of bitter constituents of natural origin. The chemical structure of regaloside A and B prompted us to study the biological activity of these glucosides. Investigation on this subject is still in progress.

EXPERIMENTAL

All melting points are uncorr. The ^1H NMR spectra were run on a Varian EM-390 (90 MHz) spectrometer, unless otherwise stated and the ^{13}C NMR spectra on a Bruker AM-400 (100.6 MHz) spectrometer with TMS as the int. standard.

Plant material. The dormant fresh bulbs of *Lilium regale* used in this investigation were purchased from Sakata-shubyoo, Japan.

Extraction and isolation. The dormant fresh bulbs of this plant (8.6 kg) were cut into pieces and extracted with hot MeOH (68 l) followed by concn. the MeOH soln to a small vol. under red. pres. The crude residue, a dark viscous syrup, was diluted with H_2O, and extracted with $CHCl_3$ and then with *n*-BuOH. The *n*-BuOH soluble phase, bitter to the taste, was repeatedly subjected to CC on silica gel with various solvent systems and on Sephadex LH-20 with MeOH as the eluent to furnish regaloside A (**1**) and B (**2**). Purification of the compounds was carried out by prep. TLC.

Regaloside A (**1**). A bitter amorphous pale-yellow powder; 16.0 g; $[\alpha]_D^{15}$ −15.2° (MeOH; *c*1.00); UV λ_{max}^{MeOH} nm (logε): 228 (4.22), 301sh (4.43), 312 (4.47); IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1700 (C=O), 1640 (C=C), 1610, 1510 (aromatic ring); EIMS 70 eV *m/z* (rel. int.): 400 $[M]^+$ (1.1), 313 (4.5), 238 (12), 221 (10), 164 (21), 147 (100), 103 (79). ^1H NMR (CD_3OD): δ7.65 (1H, *d*, *J* = 16.0 Hz, H-7′), 7.43 (2H, *d*, *J* = 8.0 Hz, H-2′, 6′), 6.81 (2H, *d*, *J* = 8.0 Hz, H-3′, 5′), 6.32 (1H, *d*, *J* = 16.0 Hz, H-8′), 4.38–3.15 (H-1–3, and H-1″–6″).

Acetylation of **1**. A soln of **1** (100 mg) and Ac_2O–pyridine was allowed to stand at room temp. for 24 hr. The crude product was chromatographed on silica gel to give colourless needles (**1a**) recrystallized from EtOH, mp 137–139° (Found: C, 55.00; H, 5.63. Calc. for $C_{30}H_{36}O_{16}$: C, 55.21; H, 5.56%). IR ν_{max}^{KBr} cm^{-1}: 1750, 1710 (C=O), 1640 (C=C), 1600, 1510 (aromatic ring); EIMS 70 eV *m/z* (rel. int.): 652 $[M]^+$ (1), 610 (8), 331 (56), 305 (84), 263 (38), 189 (35), 169 (100), 147 (88), 109 (35); ^1H NMR ($CDCl_3$): δ7.65 (1H, *d*, *J* = 16.0 Hz, H-7′), 7.52 (2H, *d*, *J* = 8.0 Hz, H-2′, 6′), 7.11 (1H, *d*, *J* = 8.0 Hz, H-3′, 5′), 6.37 (1H, *d*, *J* = 16.0 Hz, H-8′), 5.40–3.57 (H-1–3, and H-2″–6″), 4.52 (1H, *d*, *J* = 7.5 Hz, H-1″), 2.28, 2.06 × 2, 2.04, 2.00, 1.98 (each 3H, *s*, OAc).

Enzymatic hydrolysis of **1**. A mixture of **1** (250 mg) and *β*-glucosidase (23 mg) was incubated at 37° for 30 hr in AcOH–NaOAc buffer (pH 5.0). The reaction mixture was diluted with H_2O and then extracted with *n*-BuOH. The organic layer was chromatographed on a silica gel column to afford colourless plates (**3**) recrystallized from MeOH–H_2O, 98 mg, mp 119–121°, $[\alpha]_D^{20}$ + 12.8° (MeOH; *c*0.60), identified as 1-*O*-*p*-coumaroylglycerol; IR ν_{max}^{KBr} cm^{-1}: 3420, 3160 (OH), 1690 (C=O), 1630 (C=C), 1610, 1590 (aromatic ring); ^1H NMR (CD_3OD): δ7.66 (1H, *d*, *J* = 16.0 Hz, H-7′), 7.44 (2H, *d*, *J* = 8.0 Hz, H-2′, 6′), 6.83 (1H, *d*, *J* = 8.0 Hz, H-3′, 5′), 6.35 (1H, *d*, *J* = 16.0 Hz, H-8′), 4.25 (2H, *m*, H-1), 3.87 (1H, *m*, H-2), 3.67 (2H, *d*, *J* = 5.5 Hz, H-3). The H_2O layer was completely evapd off under red. pres. and the resulting residue was converted to the TMSi derivative for GC examination (OV-17 G-SCOT 20 m; 180°; N_2, 1 ml/min; FID), and identified as D-glucose.

Acetylation of **3**. To a pyridine soln of **3** (50 mg) was added Ac_2O–pyridine and it was left standing overnight. Work-up as usual gave the pure acetate (69 mg) as a colourless viscous syrup, $[\alpha]_D^{20}$ − 12.4° ($CHCl_3$; *c*1.50); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (C=O), 1640 (C=C), 1610, 1590, 1510 (aromatic ring); ^1H NMR ($CDCl_3$) δ7.67 (1H, *d*, *J* = 16.0 Hz, H-7′), 7.51 (2H, *d*, *J* = 8.0 Hz, H-2′, 6′), 7.10 (2H, *d*, *J* = 8.0 Hz, H-3′, 5′), 6.38 (1H, *d*, *J* = 16.0 Hz, H-8′), 5.33 (1H, *m*, H-2), 4.55–4.05 (4H, overlapping, H-1, 3), 2.30, 2.12, 2.10 (each 3H, *s*, OAc).

Alkaline methanolysis followed by acid hydrolysis of **1**. Compound **1** (410 mg) was treated with 3% NaOMe–MeOH at room temp. for 2 hr. The reaction soln was passed through a cation exchange resin (Amberlite IR-120B) and the eluate was concd to give a residue, which was subjected to silica gel column chromatography, yielding methyl *p*-coumarate (121 mg) and glycerol glucoside (**4**) (107 mg). Methyl *p*-coumarate; colourless needles recrystallized from H_2O–MeOH, mp 134–136°; IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1680 (C=O), 1640 (C=C), 1600, 1590, 1520 (aromatic ring); ^1H NMR ($CDCl_3$): δ7.65 (1H, *d*, *J* = 16.0 Hz, H-7), 7.43 (each 2H, *d*, *J* = 8.0 Hz, H-2, 6), 6.89 (2H, *d*, *J* = 8.0 Hz, H-3, 5), 6.38 (1H, *d*, *J* = 16.0 Hz, H-8), 5.95 (1H, *br s*, Ar-OH), 3.81 (3H, *s*, COOMe). Glycerol glucoside (**4**); colourless crystalline powder, $[\alpha]_D^{26}$–29.8° (H_2O; *c*0.26); IR ν_{max}^{KBr} cm^{-1}: 3350 (OH); ^1H NMR (Bruker AM-400, 400 MHz, C_5D_5N): δ5.00 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.58–3.93 (11H, H-1–3, and 2′–6′). Compound **4** (25 mg) in 10% HCl (2 ml) was refluxed for 2 hr to afford D-glucose and glycerol, converted to the tetramethylsilane ether and identified by a direct GC comparison with authentic samples (OV-17 G-SCOT 20 m; 180° for D-glucose, 100° for glycerol; N_2; 1 ml/min; FID).

Acetylation of **4**. Glycerol glucoside (**4**) was acetylated with Ac_2O–pyridine to give the hexaacetate (**4a**) as colourless needles recrystallized from EtOH, mp 107–109° (Found: C, 49.76; H, 6.01. Calc. for $C_{21}H_{30}O_{14}$: C, 49.80; H, 5.97%); $[\alpha]_D^{27}$ − 5.0° ($CHCl_3$; *c*0.21); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (C=O), 1370, 1230; ^1H NMR (Bruker AM-400, 400 MHz, $CDCl_3$): δ5.20 (1H, *dd*, *J* = 9.5,

9.5 Hz, H-3′), 5.19 (1H, *m*, H-2), 5.08 (1H, *dd*, *J* = 9.5, 9.5 Hz, H-4′), 4.98 (1H, *dd*, *J* = 9.5, 7.9 Hz, H-2′), 4.53 (1H, *d*, *J* = 7.9 Hz, H-1′), 4.30 (1H, *dd*, *J* = 12.1, 3.6 Hz, H-1a), 4.25 (1H, *dd*, *J* = 12.3, 4.8 Hz, H-6′a), 4.14 (1H, *dd*, *J* = 12.3, 2.5 Hz, H-6′b), 4.13 (1H, *dd*, *J* = 12.1, 6.1 Hz, H-1b), 3.95 (1H, *dd*, *J* = 11.0, 5.1 Hz, H-3a), 3.70 (1H, *ddd*, *J* = 9.5, 4.8, 2.5 Hz, H-5′), 3.69 (1H, *dd*, *J* = 11.0, 5.4 Hz, H-3b), 2.10, 2.08, 2.07, 2.06, 2.03, 2.01 (each 3H, *s*, OAc). This compound was shown to be identical with lilioside C hexaacetate [6] by TLC, ^{1}H NMR ($CDCl_3$) and mmp.

Periodate oxidation study of **4**. Glycerol glucoside (**4**) (31.1 mg) was dissolved in 0.01 M aq. $NaIO_4$ soln (100 ml) and the reaction mixture was allowed to stand at room temp. in the dark for 24 hr. The reaction mixture was submitted to the following tests. The HCO_2H produced was titrated with 0.01 N NaOH soln using methyl red as the indicator after the excess $NaIO_4$ was decomposed by ethylene glycol. The degree of $NaIO_4$ consumption was determined by the Fleury–Lange method [18]. To the $NaIO_4$ reaction solution was added satd $NaHCO_3$ soln, 0.01 N $NaAsO_2$ soln and 20% KI soln. After being set aside 15 min in the dark, the excess $NaAsO_2$ was titrated against 0.01 N I_2 soln using starch reagent as the indicator. A blank value was obtained from the original $NaIO_4$ soln without the sample. The HCHO produced was assayed by the Chromotropic acid method [19]. After the excess $NaIO_4$ was reduced by $NaAsO_2$ in H_2SO_4 acidic condition, the HCHO solution was mixed with chromotropic acid reagent, heated for 30 min and after cooling, the absorption of the HCHO sample was measured against that of the blank at 570 nm. The standard curve of HCHO was obtained from a known concentration of D-erythritol which had been treated by the same procedure as above. The results were as follows: 3.04 mol equivalent of $NaIO_4$ was consumed, and 0.91 mol equiv. of HCHO and 0.87 mol equivalent of HCO_2H were produced.

Regaloside B (**2**). A bitter pale-yellow amorphous powder; 5.5 g; $[\alpha]_D^5$ −21.2° (MeOH; *c*1.00); UV λ_{max}^{MeOH} nm (log ε): 225 (4.27), 301sh (4.47), 312 (4.51); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1720 (C=O), 1637 (C=C), 1610, 1590, 1520 (aromatic ring); EIMS 70 eV *m/z* (rel. int.): 442 $[M]^+$ (0.5), 330 (4.2), 280 (3.5), 263 (5.5), 205 (38), 187 (35), 163 (27), 159 (30), 147 (75), 145 (100), 117 (83), 103 (91); ^{1}H NMR (CD_3OD): δ7.62 (1H, *d*, *J* = 16.0 Hz, H-7′), 7.43 (2H, *d*, *J* = 9.0 Hz, H-2′, 6′), 6.85 (2H, *d*, *J* = 9.0 Hz, H-3′, 5′), 6.35 (1H, *d*, *J* = 16.0 Hz, H-8′), 4.48 (1H, *d*, *J* = 7.0 Hz, H-1″), 4.40–3.06 (H-1–3, and H-2″–6″), 2.01 (3H, *s*, OAc).

Acetylation of **2**. Upon acetylation of **2** (100 mg) with Ac_2O–pyridine, 130 mg of the peracetate (**2a**) was obtained as colourless needles recrystallized from EtOH, mp 118–120° (Found: C, 55.14; H, 5.58. Calc. for $C_{30}H_{36}O_{16}$: C, 55.21; H, 5.56%); IR ν_{max}^{KBr} cm^{-1}: 1750 (C=O), 1640 (C=C), 1610, 1520 (aromatic ring); EIMS 70 eV *m/z* (rel. int.): 652 $[M]^+$ (1), 610 (9), 331 (50), 305 (86), 263 (29), 189 (42), 169 (100), 147 (89), 109 (29); ^{1}H NMR ($CDCl_3$): δ7.67 (1H, *d*, *J* = 16.0 Hz, H-7′), 7.55 (2H, *d*, *J* = 8.5 Hz, H-2′, 6′), 7.12 (2H, *d*, *J* = 8.5 Hz, H-3′, 5′), 6.37 (1H, *d*, *J* = 16.0 Hz, H-8′), 5.35–3.55 (H-1–3, and H-2″–6″), 4.67 (1H, *d*, *J* = 7.5 Hz, H-1″), 2.30, 2.07 × 2, 2.00, 1.97 × 2 (each 3H, *s*, OAc).

Enzymatic hydrolysis of **2**. Hydrolysis of **2** (250 mg) with β-glucosidase (23 mg) in AcOH–NaOAc buffer (pH 5) was carried out at 37° for 30 hr. The reaction mixture was partitioned with EtOAc and H_2O. From the EtOAc layer, 1-*O*-*p*-coumaroyl-3-*O*-acetylglycerol (**5**) was obtained, and from the H_2O layer, D-glucose was detected. Compound **5**; $[\alpha]_D^{20}$ −1.8° ($CHCl_3$–MeOH = 1 : 1; *c* 0.70); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (OH), 1720 (C=O), 1640 (C=C), 1610, 1590, 1520 (aromatic ring); ^{1}H NMR ($CDCl_3$–CD_3OD = 1:1): δ7.62 (1H, *d*, *J* = 16.0 Hz, H-7′), 7.40 (2H, *d*, *J* = 8.5 Hz, H-2′, 6′), 6.80 (2H, *d*, *J* = 8.5 Hz, H-3′, 5′), 6.29 (1H, *d*, *J* = 16.0 Hz, H-8′), 4.40–4.05 (5H, overlapping, H-1, 2, 3), 2.05 (3H, *s*, OAc). D-glucose was identified by a direct GC comparison with authentic sample.

Alkaline methanolysis followed by acid hydrolysis of **2**. Compound **2** (308 mg) was treated with 3% NaOMe–MeOH at room temp. for 2 hr, and the similar work-up as in the case of **1** yield methyl *p*-coumarate (98 mg) and glycerol glucoside (**6**) (83 mg). Glycerol glucoside (**6**); colourless needles recrystallized from EtOH, mp 160–162°, $[\alpha]_D^{26}$ −30.6° (H_2O; *c*0.22); IR ν_{max}^{KBr} cm^{-1}: 3370 (OH); ^{1}H NMR (Bruker AM-400, 400 MHz, C_5D_5N): δ5.20 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.58–3.96 (11H, H-1–3, and 2′–6′). Compound **6** was hydrolysed with 10% HCl (2 ml) and the presence of D-glucose and glycerol was shown by GC examination.

Periodate oxidation study of **6**. Glycerol glucoside (**6**) (22.3 mg) was subjected to the oxidation test as for **4**. The results were as follows: 1.99 mol equivalent of $NaIO_4$ was consumed, and 0.92 mol equiv. of HCO_2H was produced. No HCHO was produced.

Acetylation of **5**. Upon acetylation of **5** with Ac_2O–pyridine, the corresponding diacetate (**5a**) was obtained as a colourless viscous syrup; $[\alpha]_D^{20}$ −11.4° ($CHCl_3$; *c*1.40); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (C=O), 1645 (C=C), 1610, 1510 (aromatic ring); ^{1}H NMR ($CDCl_3$): δ7.68 (1H, *d*, *J* = 16.0 Hz, H-7′), 7.55 (2H, *d*, *J* = 8.5 Hz, H-2′, 6′), 7.13 (2H, *d*, *J* = 8.5 Hz, H-3′, 5′), 6.40 (1H, *d*, *J* = 16.0 Hz, H-8′), 5.35 (1H, *m*, H-2), 4.55–4.05 (4H, overlapping, H-1, 3), 2.30, 2.10, 2.08 (each 3H, *s*, OAc).

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