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PHENOLIC GLYCOSIDES FROM *LILIUM LONGIFLORUM*

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Key Word Index—*Lilium longiflorum*; Liliaceae; feruloyl sucrose; bitter principle; growth inhibitory effect; lettuce seedling test.

Abstract—Three bitter principles were isolated from the bulb scales of *Lilium longiflorum* and identified as 3,6′-diferuloylsucrose, 4-acetyl-3,6′-diferuloylsucrose and 3-feruloyl-4-acetyl-6′-(13′-*O*-β-D-glucopyranosyl)feruloylsucrose on the basis of chemical and spectral data. The growth inhibitory effects of these compounds have been investigated by the lettuce seedling test.

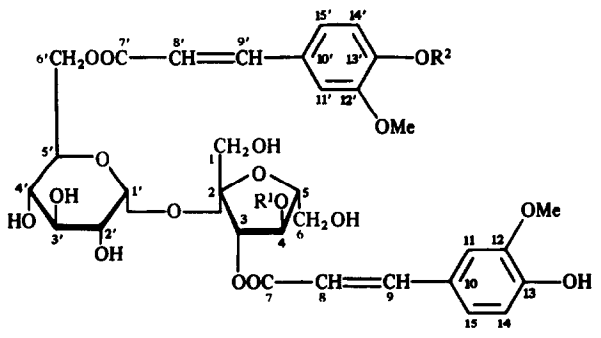
INTRODUCTION

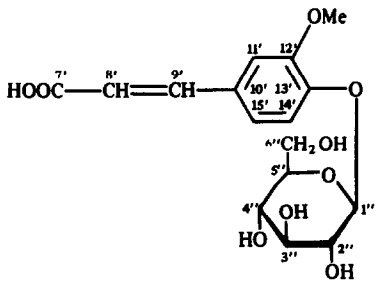
In the continuing studies of growth inhibitors [1–3], we have found 2,3-dihydroxy-3-*O*-*p*-coumaryl-1,2-propanedicarboxylic acid [3] as an inhibitory principle in *Lilium longiflorum*. On further investigation of this plant, we have isolated three bitter substances from the bulb scales. This paper describes the isolation, identification and structural elucidation of these compounds and these growth inhibitory effects have been tested using lettuce seedlings.

RESULT AND DISCUSSION

The acetone extract of *L. longiflorum* bulb scales was partitioned with organic solvents and repeated chromatography on Sephadex LH-20 using water–methanol to give 1–3. Compounds 1 and 2 were identified as 3,6′-diferuloylsucrose[4] and 4-acetyl-3,6′-diferuloylsucrose [5], respectively, by comparison of ^{1}H NMR [4, 5] and ^{13}C NMR [6–8] data. The proton signals of the sucrose moiety of 2 were assigned by proton spin decoupling experiments using a 500 MHz NMR spectrometer (Table 1).

Compound 3 on enzymatic hydrolysis with naringinase, gave 2 which was identified by ^{13}C NMR and FDMS, whereas alkaline hydrolysis gave sucrose, ferulic acid and the phenolic glycoside 4. The FDMS of 4 suggested that 4 was a feruloyl glucoside from the molecular ion at *m/z* 356 and fragments at *m/z* 194 (feruloy) and *m/z* 163 (glucosyl). Furthermore, enzymic hydrolysis of 4 with β-glucosidase afforded glucose and ferulic acid. From the above results it is clear that 3 is a glucoside of 2. The ^{1}H NMR spectrum of 3 resembled that of 2 except for a new signal due to an anomeric proton at δ4.98 (*d*, *J* = 7.3 Hz) and other proton signals due to H-5″ (δ4.03) and H-6″ (δ3.66) corresponding to a terminal glucose moiety. The position of the terminal glucose moiety was also assumed from a comparison of the ^{1}H NMR spectra of 3 and 2. Thus, the signal for H-6′ in 3 was found to have shifted less (0.03 ppm) than that in 2 while the individual signal due to H-3 was identical in 2 and 3. This suggested that the terminal glucose is linked to the hydroxyl group of the feruloyl moiety, which is attached to the C-6′ hydroxyl of the sucrose moiety. The ^{13}C signals of 3 were confirmed by measurement of the ^{1}H-^{13}C shift correlated spectrum as shown in the exper-





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1 $R_1 = R_2 = H$
2 $R_1 = Ac$, $R_2 = H$
3 $R_1 = Ac$, $R_2 = Glc$

Table 1 ^{1}H NMR chemical shifts (500 MHz; DMSO-d_6) of feruloyl ester, 2,3 and 1 peracetate

	2	3*	1-Peracetate†
H-1	3.36, *br s*	3.31, *d*, *J* = 11.0	–
		3.34, *d*, *J* = 11.0	
H-3	5.60, *d*, *J* = 7.0	5.60, *d*, *J* = 7.0	5.67, *d*, *J* = 5.9
H-4	5.40, *t*, *J* = 7.0	5.41, *t*, *J* = 7.0	5.54, *dd*, *J* = 5.9, 5.9
H-5	4.03, *dd*, *J* = 6.7, 12.1	4.03, *dd*, *J* = 6.4, 12.7	–
H-6	3.66, *dd*, *J* = 4.3, 12.1	3.66, *dd*, *J* = 6.4, 11.8	–
	3.72, *dd*, *J* = 7.1, 12.1	3.74, *dd*, *J* = 4.0, 11.8	–
H-1′	5.26, *d*, *J* = 3.5	5.26, *d*, *J* = 3.7	5.78, *d*, *J* = 3.7
H-2′	3.30, *dd*, *J* = 3.5, 9.2	3.30, *dd*, *J* = 2.7, 9.0	4.95, *dd*, *J* = 3.7, 10.3
H-3′	3.19, *t*, *J* = 9.2	3.17, *t*, *J* = 9.0	5.50, *t*, *J* = 9.6
H-4′	3.49, *t*, *J* = 9.2	3.50, *t*, *J* = 9.0	5.54, *t*, *J* = 9.6
H-5′	4.07, *dd*, *J* = 6.4, 12.8	4.07, *dd*, *J* = 7.0, 9.0	–
H-6′	4.16, *dd*, *J* = 6.4, 11.9	4.19, *dd*, *J* = 7.0, 11.9	–
	4.44, *d*, *J* = 11.9	4.45, *d*, *J* = 11.9	
H-1″		4.98, *d*, *J* = 7.3	
H-2″		3.90, *dd*, *J* = 2.7, 9.0	
H-3″		3.42–3.48, *m*	
H-4″		3.17, *t*, *J* = 9.2	
H-5″		4.03, *dd*, *J* = 6.4, 11.8	
H-6″		3.66, *dd*, *J* = 4.0, 11.9	
H-8, 8′	6.39, *d*, *J* = 15.9	6.39, *d*, *J* = 15.9	6.47, *d*, *J* = 16.0
	6.51, *d*, *J* = 15.9	6.61, *d*, *J* = 15.9	6.51, *d*, *J* = 16.2
H-9, 9′	7.54, *d*, *J* = 15.9	7.58, *d*, *J* = 15.9	7.36, *d*, *J* = 16.2
	7.61, *d*, *J* = 15.9	7.60, *d*, *J* = 15.9	7.73, *d*, *J* = 16.2
H-11, 11′	7.27, *d*, *J* = 1.8	7.28, *d*, *J* = 1.8	7.29, *d*, *J* = 1.8
	7.28, *d*, *J* = 1.8	7.33, *d*, *J* = 1.8	7.17, *d*, *J* = 1.8
H-14, 14′	6.79, *d*, *J* = 8.2	6.79, *d*, *J* = 7.9	7.04, *d*, *J* = 8.3
	6.80, *d*, *J* = 8.2	7.10, *d*, *J* = 7.9	7.05, *d*, *J* = 8.3
H-15, 15′	7.09, *dd*, *J* = 1.8, 8.2	7.14, *dd*, *J* = 1.8, 8.2	7.13, *dd*, *J* = 1.8, 8.4
	7.15, *dd*, *J* = 1.8, 8.2	7.14, *dd*, *J* = 1.8, 8.2	7.18, *dd*, *J* = 1.8, 8.4
MeCO	1.97	1.98	1.89, 1.97, 2.08, 2.10, 2.11, 2.12, 2.32 (×2)
MeO	3.80, 3.81	3.80, 3.81	3.89, 3.90

*Confirmed by measurement of the ^{1}H–^{13}C shift-correlated spectrum.
†In $CDCl_3$.

imental. On the basis of the above evidence, the structure of **3** has been established as 3-feruloyl-4-acetyl-6′(13′-*O*-β-D-glucopyranosyl)-feruloylsucrose and named as lilongiside.

The effects of **1**, **2** and **3** on the lettuce seedling growth test have been investigated. The growth rate of the hypocotyl of the seedling compared with the control is shown in Fig. 1. The inhibitory effects of **1**, **2** and **3** are not so strong in lower concentration, but **2** and **3** are clearly dose-dependent. Compound **3** inhibited the growth rate of hypocotyl by 5% at 10 ppm of **3**, 21% at 100 ppm, 39% at 500 ppm and 78% at 1000 ppm. On the other hand, **1**, **2** and **3** have no inhibitory effect on root growth. We previously reported that the coumaroylester of 2,3-dihydroxy-1,2-propanedicarboxylic acid isolated from the acidic fraction, significantly inhibited the growth of the root more than that of the hypocotyl. From these results, it is reasonable to speculate that the inhibitory mechanism of the feruloyglycosides **1**–**3** and the coumaroylester of the dicarboxylic acid are different. However, it is not clear whether the difference is due to the liberation of *p*-coumaric and ferulic acids from individual esters or not.

A number of cinnamic acid esters with sucrose have been reported [4–8]. More recently, Nakano *et al.* [9] isolated some feruloyl esters of sucrose in which the position of the acyl group in the molecules was different from those in *L. longiflorum* from another number of the

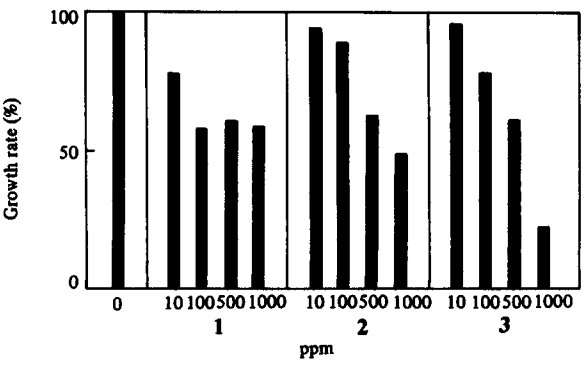

Fig. 1. Effect of 3,6′-diferuloylsucrose (**1**), 4-acetyl-3,6′-diferuloylsucrose (**2**) and lilongiside (**3**) on the growth of lettuce seedling hypocotyls.

Liliaceae, *Heloniopsis orientalis*. The distribution of such sucrose esters in the Liliaceae may prove to be of some taxonomic interest.

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR and ^{13}C NMR: TMS as an int. standard and chemical shifts were expressed in ppm and coupling constants (J) in Hz. ^{1}H NMR spectrum; JEOL PS-100(100 MHz) and JEOL GX-500 (500 MHz) spectrometer, ^{13}C NMR spectrum; JEOL FX-100 spectrometer; TLC; Kiesel gel 60 F 254 plate, developed with *n*-BuOH–HOAc–H_2O (4:1:5), $CHCl_3$–MeOH–H_2O (5:3:1).

Bioassay method. The lettuce seedling growth test was done as previously described [10] with modification.

Isolation of **1–3**. Fresh bulb scales (10 kg) were mechanically homogenated with Me_2CO (4 l). After filtration the filtrate was evaporated to almost dryness. The residue (12.3 g) was suspended in H_2O and partitioned with Et_2O, EtOAc and *n*-BuOH. The *n*-BuOH soluble fraction (2.65 g) was repeatedly chromatographed on Sephadex LH-20 (25–100 μm) using H_2O–MeOH as an eluant to give **1** (130 mg), **2** (80 mg) and **3** (68 mg).

3,6′-Diferuloylsucrose (**1**). Compound **1** was obtained as an amorphous powder. $[\alpha]_D^{20}$ +85.0° (MeOH; *c* 1.1), UV λ_{max}^{MeOH} nm (log ε): 300 (4.58), 325 (4.63), IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1700 (CO), 1630, 1590 (C=C), FDMS *m/z*: 716 (M + Na)$^+$ (Found: C, 54.89; H, 5.48. $C_{32}H_{38}O_{17}$. 1/2 H_2O requires; C, 54.62; H, 5.59), ^{13}C NMR (in pyridine-d_5) 65.57 (C-1), 104.53 (C-2), 79.34 (C-3), 73.89 (C-4), 84.55 (C-5), 63.28 (C-6), 92.58 (C-1′), 72.95 (C-2′) 75.06 (C-3′), 72.07 (C-4′), 71.54 (C-5′), 64.98 (C-6′), 166.52, 167.40 (C-7, 7′), 114.61, 116.37, (C-8, 8′), 145.39, 145.95 (C-9, 9′), 123.52, 124.27 (C-10, 10′), 111.03, 111.80 (C-11, 11′), 148.59, 149.24 (C-12, 12′), 150.35, 150.64 (C-13, 13′), 114.96, 116.37 (C-14, 14′), 122.34, 123.16 (C-15, 15′), 55.78 (Me-O), ^{1}H NMR (500 MHz in DMSO-d_6) 3.16 (1H, *t*, *J* = 9.3, H-3′), 3.30 (1H, *dd*, *J* = 3.7, 9.2, H-2′), 3.36 (2H, *br s*, H-1), 3.46 (1H, *t*, *J* = 9.3, H-4′), 3.62 (1H, *dd*, *J* = 4.3, 11.6, H-6), 3.67 (1H, *dd*, *J* = 7.0, 11.6, H-6), 4.07 (1H, *dd*, *J* = 7.0, 11.6, H-5), 4.07 (1H, *dd*, *J* = 9.2, 9.5, H-5′), 4.17 (1H, *dd*, *J* = 4.6, 11.8, H-6′), 4.21 (1H, *dd*, *J* = 8.0, 8.0, H-4), 4.44 (2H, *d*, *J* = 11.8, H-6′), 5.27 (1H, *d*, *J* = 3.7, H-1′), 5.39 (1H, *d*, *J* = 7.9, H-3).

Akaline hydrolysis of **1**. Compound **1** (10 mg) was treated with 1N NaOH under N_2 at 50° for 30 min. The reaction product was passed through Amberlite IR-120, washed with H_2O and the eluate extracted with Et_2O. The H_2O soluble fraction subjected to CC on Sephadex LH-20 using H_2O to give sucrose (3.5 mg), identified by ^{13}C NMR comparison with an authentic sample. Ferulic acid (4 mg) cryst. from the Et_2O soluble fraction, mp 172–174°.

Acetylation of **1**. Compound **1** (30 mg) was treated with Ac_2O–pyridine (0.5 mg) at room temp. overnight, diluted with H_2O and extracted ×3 with EtOAc. The dried EtOAc extract was chromatographed on silica gel 60 using C_6H_6–Me_2CO (5:2) as an eluant to give the peracetate of **1** (35.6 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (CO), 1630, 1595 (C=C), FD-MS *m/z*: 507.1488 $(C_{24}H_{27}O_{12})^+$, 483 $(C_{22}H_{27}O_{12})^+$, 464.1272 $(C_{22}H_{24}O_{11})^+$, 289.0909 $(C_{12}H_{17}O_8)^+$ (Found: C, 55.93; H, 5.28. $C_{48}H_{54}O_{25}$ requires: C, 55.92; H, 5.28%), ^{13}C NMR (in CD_3Cl) 63.65 (C-1), 103. 96 (C-2), 78.98 (C-3), 76.20 (C-4), 75.00 (C-5), 63.65 (C-6), 90.10 (C-1′), 69.50 (C-2′), 70.09 (C-3′), 69.56 (C-4′, 5′), 62.13 (C-6′), 165.32, 166.33 (C-7, 7′), 116.42, 117.47 (C-8, 8′), 144.85, 146.31 (C-9, 9′), 132.92, 133.15 (C-10, 10′), 111.39 (C-11, 11′), 141.52, 141.87 (C-12, 12′), 151.35, 151.46 (C-13, 13′), 121.57, 121.92 (C-14, 14′), 123.15 (C-15, 15′), 20.59 (Me-C), 55.93 (Me-O), 168.55, 169.54, 169.78, 169.95, 170.24, 170.42 (Me–CO), ^{1}H NMR ($CDCl_3$): see Table 1.

4-Acetyl-3,6′-diferuloylsucrose (**2**). Compound **2** was obtained as an amorphous powder, $[\alpha]_D^{20}$ +74.6° (MeOH *c* 1.1), $FeCl_3$ test: bluish grey, diazotized benzidine test: orange, UV λ_{max}^{MeOH} nm (log ε): 298 (4.66), 326 (4.71), IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1710 (CO), 1635, 1600 (OH), FDMS *m/z*: 776 (M+K)$^+$ 759 (M + Na)$^+$, 736 (M)$^+$, 693 (M − Ac)$^+$ (Found: C, 54.60; H, 5.42. $C_{34}H_{40}O_{18}$. 1/2 H_2O requires: C, 54.76; H, 5.54%), ^{1}H NMR (DMSO-d_6) see Table 1, ^{13}C NMR (pyridine-d_5): 63.11 (C-1), 105.59 (C-2), 76.93 (C-3, 4), 82.97 (C-5), 63.11 (C-6), 93.16 (C-1′), 72.89 (C-2′), 74.82 (C-3′), 71.98 (C-4′), 72.42 (C-5′), 64.80 (C-6′), 165.99, 167.46 (C-7, 7′), 114.20, 116.37 (C-8, 8′), 145.31, 146.48 (C-9, 9′), 123.45, 124.22 (C-10, 10′), 111.21, 111.80 (C-11, 11′), 148.24, 149.35 (C-12, 12′), 150.41, 150.64 (C-13, 13′), 115.13, 115.66 (C-14, 14′), 122.28, 123.34 (C-15, 15′), 20.68 (Me-CO), 55.78 (Me-O), 169.92 (Me-CO).

Compound **2** *octa-acetate.* Compound **2** was acetylated and treated as for **1** to give the octa-acetate of **2** which was identical with peracetate of **1** (^{1}H NMR and ^{13}C NMR).

3-Feruloyl-4-acetyl-6′(13′-O-β-D-glucopyranosyl)feruloyl-sucrose (**3**). Compound **3** was obtained as an amorphous powder. $[\alpha]_D^{26}$ +79.4° (MeOH; *c* 1.0), $FeCl_3$ test: bluish grey, UV λ_{max}^{MeOH} nm (log ε): 296 (4.37), 321 (4.40), IR λ_{max}^{KBr} cm^{-1}: 3422 (OH), 1703 (CO), 1632, 1597 (C=C), FD-MS *m/z*: 921 (M + Na)$^+$, 879 (M + Na − Ac)$^+$ 759 (M + Na − glc)$^+$, (Found: C, 52.04; H, 5.62. $C_{40}H_{50}O_{23}$.H_2O requires: C, 52.40; H, 5.72%), ^{1}H NMR (DMSO-d_6): see Table 1, ^{13}C NMR (pyridine-d_5): 64.80 (C-1), 105.63 (C-2), 76.91 (C-3, 4), 82.93 (C-5), 63.10 (C-6), 93.17 (C-1′), 72.87 (C-2′), 74.51 (C-3′), 71.47 (C-4′), 72.40 (C-5′), 64.80 (C-6′), 101.48 (C-1″), 74.80 (C-2″), 78.12 (C-3″), 71.00 (C-4″), 78.25 (C-5″), 62.11 (C-6″), 166.44, 167.61 (C-7, 7′), 114.58, 117.00 (C-8, 8′), 145.03, 146.84 (C-9, 9′), 126.37, 129.06 (C-10, 10′), 111.74, 112.09 (C-11, 11′), 150.12 (C-12, 12′), 151.11 (C-13, 13′), 115.83, 116.65 (C-14, 14′), 122.91, 123.56 (C-15, 15′), 20.77 (Me-CO), 55.99 (Me-O), 170.36 (Me-CO).

Enzymatic hydrolysis of **3**. Compound **3** (70 mg) in acetate buffer (pH 4.5; 25 ml) was incubated with naringinase (20 mg) at 37° for 48 hr. After evapn of H_2O, the residue was chromatographed on Sephadex LH-20 using H_2O–MeOH (10:1–0:1). The MeOH eluate was evaporated to give **2** (26 mg) identified by the comparison of ^{13}C NMR and glucose which was identified by PC (R_f 0.13, in *n*-BuOH–HOAc–H_2O (4:1:5).

Akaline hydrolysis of compound **3**. Compound **3** (50 mg) was treated with 1 N NaOH as for **1** to give **4** (10.5 mg), sucrose and ferulic acid. Compound **4** was obtained as colourless needles, mp 226–228° (EtOH). ^{1}H NMR (CD_3OD): 3.84 (3H, *s*, OMe), 4.98 (1H, *d*, *J* = 8 Hz), 6.36 (1H, *d*, *J* = 16 Hz), 7.18 (1H × 2, *d*, *J* = 6 Hz), 7.15 (1H, *s*), 7.56 (1H, *d*, *J* = 16 Hz), FDMS *m/z*: 356 (M)$^+$, 194 (ferulic acid)$^+$, 163 (glucosyl)$^+$, (Found: C, 53.85; H, 5.72. $C_{16}H_{20}O_9$ requires: C, 53.93; H, 5.26%).

Enzymatic hydrolysis of **4**. Compound **4** (2 mg) was incubated with β-glucosidase in acetate buffer (pH 4.5) at 37° for 48 hr. The incubated solution was tested for glucose (PC; R_f 0.13 in *n*-BuOH–HOAc–H_2O (4:1:5) and ferulic acid (TLC; R_f 0.79 in $CHCl_3$–MeOH–HOAc (7:3:1).

Acetylation of **3**. Compound **3** (10 mg) was acetylated and treated by the same manner as **1** to give the undeca-acetate of **3** (12 mg). EIMS *m/z* (ret. int.) 507 (5.8), 464 (9.2), 331 (19.6), 169 (100), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (CO), 1637, 1600 (C=C), ^{1}H NMR ($CDCl_3$; 100 MHz): 1.85 (Ac), 2.04 (Ac × 2), 2.08 (Ac × 4), 2.12 (Ac), 2.32 (Ac × 2), 3.84 (OMe), 3.85 (OMe), 6.40 (1H, *d*, *J* = 16 Hz), 6.52 (1H, *d*, *J* = 16 Hz), 7.00–7.20 (4H, *m*), 7.34 (2H, *s*), 7.59 (1H, *d*, *J* = 16 Hz), 7.72 (1H, *d*, *J* = 16 Hz) (Found: C, 54.72; H, 5.39. $C_{61}H_{70}O_{34}$ requires: C, 54.38; H, 5.24).

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