Novel Phenolic Amide Derivative from the Bulbs of *Lilium regale*

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A novel phenolic amide derivative, (±)-*N*-(4-methylsuccinimido-*n*-butyl)-*p*-coumaramide, was isolated from the bulbs of *Lilium regale*. The structure was elucidated by extensive spectral studies and hydrolysis. 3,6′-*O*-Diferuloylsucrose, which has been detected in the anthers and bulbs of several plants of the Liliaceae, was also isolated and identified.

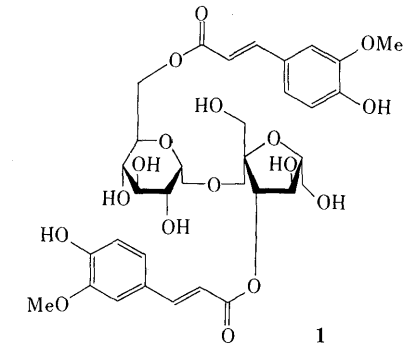
Keywords *Lilium regale*; Liliaceae; phenolic glycoside; phenoic amide; *p*-coumaric acid; putrescine; methylsuccinic acid; COLOC spectrum; bulb

Lilium regale (Liliaceae) is a perennial plant native to China, and is classified on the basis of the flower shapes into the subgenus *Leucolilion*.[1] The bulbs are significantly bitter to the taste and not edible. Dried bulbs of *Lilium* spp. are used in traditional Chinese medicines for the treatment of cough and cerebral excitation.[2] Some publications have indicated that the more bitter bulbs are of better quality, when used as a medicinal material.[3] The structural elucidation of two novel bitter phenylpropanoid glycerol glucosides isolated from bulbs of *L. regale* and designated as regalosides A and B has been discussed in an earlier communication.[4] In our systematic survey of the ingredients of the bulbs of the genus *Lilium*,[5] we have now undertaken a reinvestigation of the methanolic extract of *L. regale*, resulting in the isolation of a phenolic glycoside (**1**) and a phenolic amide (**2**), the latter of which has not been found previously in plants. This paper mainly concerns the structure of the new amide.

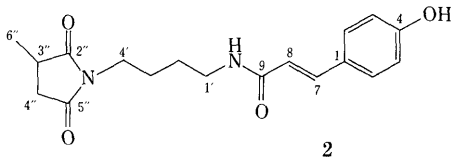
The methanol extract of the bulbs of *L. regale* was further fractionated by silica gel and Sephadex LH-20 column chromatographies after isolation of regalosides A and B[4] to give **1** and **2**.

Compound **1** was obtained as a pale-yellow amorphous powder and revealed a bitter taste. The structure was shown to be 3,6′-*O*-diferuloylsucrose based on the infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra, and a direct thin-layer chromatography (TLC) comparison with an authentic sample.[5a]

Compound **2** was obtained as colorless needles, recrystallized from methanol, mp 184.0—185.5 °C. The molecular formula of **2**, $C_{18}H_{22}N_2O_4$ was confirmed from the molecular ion peak of the high-resolution, electron impact mass spectrum (EI-MS), *m/z* 330.1579 (Calcd: 330.1581) and elemental analysis (Calcd: C, 65.44; H, 6.71; N, 8.48. Found: C, 64.76; H, 6.73; N, 8.38). Treatment of **2** with acetic anhydride in pyridine yielded a monoacetate (**2a**). The EI-MS of **2a** showed a molecular ion peak at *m/z* 372.1721 (Calcd for $C_{20}H_{24}N_2O_5$: 372.1686) and the ^{1}H-NMR spectrum exhibited a new aromatic acetoxyl group at δ 2.30 (3H, s). The existence of a *p*-coumaroyl moiety in **2** was indicated by the ultraviolet (UV) absorption bands (λ_{max} 225, 293 and 308 nm, bathochromic shifts at 308 to 353 nm upon addition of sodium methoxide reagent were observed), a prominent fragment ion peak at *m/z* 147 in the EI-MS, and signals due to four aromatic protons as an A_2B_2 system (δ 7.53 and 7.11, each 2H, d, J=8.4 Hz) and olefinic protons as an AM system (δ 8.06 and 6.80, each 1H, d, J=15.6 Hz, a *trans* arrangement) in the ^{1}H-NMR spectrum. The ^{1}H-NMR spectrum also revealed four protons (δ 3.56) attributable to two methylene groups adjacent to amino groups and four additional protons (δ 1.69) corresponding to the other methylene groups of 1,4-diaminobutane. The remaining ^{1}H-NMR signals [δ 2.87 (1H, dd, J=16.2, 8.8 Hz), 2.82 (1H, m), 2.30 (1H, dd, J=16.2, 3.4 Hz) and 1.21 (3H, d, J=6.6 Hz)] were assigned to CH_3–CH–CH_2 groups confirmed by double resonance experiments. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, signals due to a *p*-coumaroyl moiety and four consecutive methylene groups between nitrogen atoms were readily recognized with the aid of a distortionless enhancement by polarization transfer (DEPT) spectrum, and in addition, a methyl (δ 16.4), a methylene (δ 36.6), a methine (δ 35.0) and two carbonyl carbon (δ 180.9 and 176.7) signals were observed. Hydrolysis of **2** by refluxing in 2 N sodium hydroxide for 4 h under a nitrogen atmosphere gave *p*-coumaric acid, methylsuccinic acid and 1,4-aminobutane, that is, putrescine. Detailed inspection of the IR spectrum of **2** provided information concerning the functional groups. The amide band A (carbonyl) was present at 1645 cm^{-1} and the amide band B at 1545 cm^{-1}. The two characteristic absorptions at 1765 and 1685 cm^{-1} (intensity, 1765 < 1685) appeared to be due to carbonyl group(s) of a five-membered cyclic imide. From the findings presented above, the possible structure of **2** seemed to be *N*-(4-methylsuccinimido-*n*-butyl)-*p*-coumaramide. Final confir-



1



2

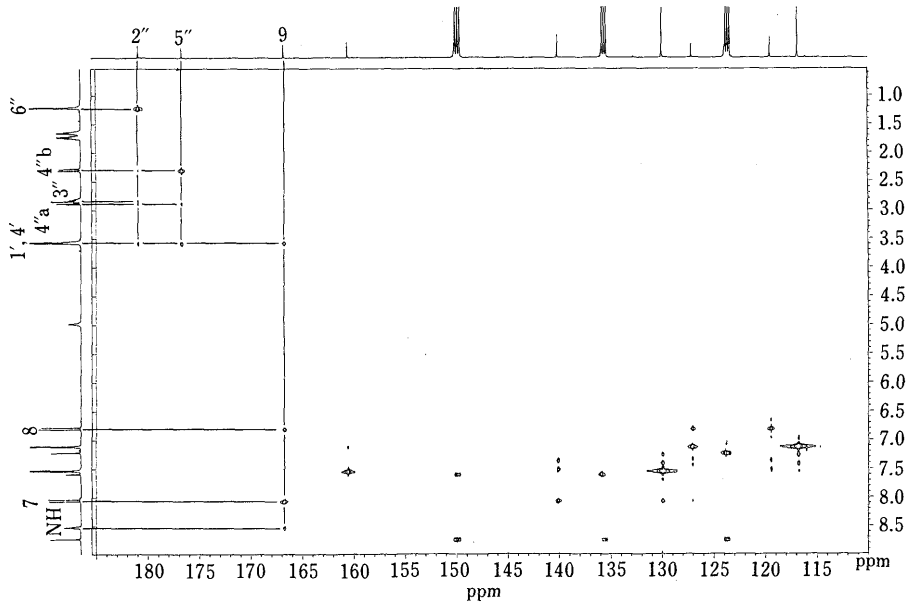


Fig. 1. ^{1}H–^{13}C Long-range Coupling Correlation 2D (COLOC) Spectrum in C_5D_5N (500 MHz)

mation of the proposed structure was obtained from the ^{1}H–^{13}C long-range coupling correlation 2D (COLOC) spectrum. Correlations between the three carbonyl carbons (δ 180.9, 176.7 and 166.7) and the $^{2}J_{CH}$ and/or $^{3}J_{CH}$ protons were observed as shown in Fig. 1. Compound **2** showed no specific rotation. Furthermore, the optical rotatory dispersion (ORD) spectrum displayed no curve as well as no Cotton effect in the 600 to 200 nm region. Compound **2** was obtained as a racemate.

Hydroxycinnamic acid conjugates with the aliphatic amines, putrescine, spermidine and spermine, are known from various plants, and conjugates of this type are especially accumulated in the reproductive organs.[6] Some of the amides are suggested to protect the plants from viral, bacterial or fungal infection and may be produced as phytoalexins.[7] *L. regale* as well as *L. henryi* and *L. hansonii* is known for its strong resistance to viral diseases,[8] which can sometimes cause fatal damage to lily plants.[9] The phenolic glycosides, **1**, regaloside A and regaloside B isolated in good yields, and **2** may protect *L. regale* from infection. Some biological tests of the isolated compounds are in progress.

Experimental

The melting point was recorded with a Yazawa micro melting apparatus and is uncorrected. Optical rotation was measured with a JASCO DIP-360 automatic polarimeter. IR spectra were recorded on a Hitachi 260-30 spectrometer, the UV spectrum on a Hitachi 557 spectrometer, MS on a Hitachi M-80 machine, and ORD on a JASCO J-6000 spectrometer. NMR spectra were taken with a Bruker AM-400 or a AM-500 spectrometer. Chemical shifts are reported in ppm (δ scale) with tetramethylsilane (TMS) as an internal standard, and the following abbreviations are used: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet; br, broad. Column chromatographies were carried out on Fuji Davison silica gel BW-300 (200—400 mesh, Fuji Davison Co., Ltd.) and Saphadex LH-20 (25—100 μm, Pharmacia Fine Chemicals Co., Ltd.). TLC was performed on precoated Kieselgel 60 F_{254} plates (0.25 mm thick, Merck), and spots were visualized under UV light (254 nm) irradiation and by spraying 10% H_2SO_4 solution followed by heating. Authentic samples, putrescine and methylsuccinic acid were purchased from Wako Pure Chemicals Co., Ltd., and *p*-coumaric acid from Aldrich Chemical Company, Inc. Elemental analysis was carried out by Shonan Analytical Center Co., Ltd.

Isolation Details of the extraction and isolation procedures were as described previously.[4] The solvent systems used in the silica gel column chromatography were $CHCl_3$–MeOH, $CHCl_3$–MeOH–H_2O, EtOAc–MeOH and EtOAc–MeOH–H_2O for **1**, and $CHCl_3$–EtOAc, $CHCl_3$–acetone and $CHCl_3$–MeOH for **2**.

3,6′-*O*-Diferuloylsucrose (1) A pale-yellow amorphous powder (2.29 g).

(±)-*N*-(4-Methylsuccinimido-*n*-butyl)-*p*-coumaramide (2) Colorless needles (MeOH) (88.0 mg), mp 184.0—185.5 °C, $[\alpha]_D^{26}$ ±0° (c=0.49, $CHCl_3$–MeOH (2:1)). *Anal.* Calcd for $C_{18}H_{22}N_2O_4$: C, 65.44; H, 6.71; N, 8.48. Found: C, 64.76; H, 6.73; N, 8.38. UV λ_{max}^{EtOH} nm (log ε): 225 (4.09), 293 (4.26), 308 (4.25). UV $\lambda_{max}^{EtOH+NaOMe}$ nm: 235, 312 sh, 353. IR ν_{max}^{KBr} cm^{-1}: 3350 (NH), 3110 (OH), 3020, 2950, 2870, 2815 (CH), 1765 and 1685 (five-membered cyclic imide, intensity 1765<1685), 1645 (secondary amide A band, C=O), 1600, 1580, 1510 (aromatic ring), 1545 (secondary amide B band, NH), 1450, 1405, 1370, 1345, 1310, 1275, 1240, 1225, 1170, 1130, 985, 830. EI-MS *m/z* (%): 330.1579 (11) [M$^+$, Calcd for $C_{18}H_{22}N_2O_4$: 330.1581], 204 (10), 183 (23), 163 (13), 147 (100), 119 (22), 91 (21), 65 (17). The ORD spectrum was measured in MeCN–MeOH (1:1). ^{1}H-NMR (C_5D_5N) δ: 11.98 (1H, br s, OH), 8.55 (1H, br t, J=5.4 Hz, NH), 8.06 (1H, d, J=15.6 Hz, H-7), 7.53 (2H, d, J=8.4 Hz, H-2, -6), 7.11 (2H, d, J=8.4 Hz, H-3, -5), 6.80 (1H, d, J=15.6 Hz, H-8), 3.56 (4H, overlapping, H-1′, -4′), 2.87 (1H, dd, J=16.2, 8.8 Hz, H-4″a), 2.82 (1H, m, H-3″), 2.30 (1H, dd, J=16.2, 3.4 Hz, H-4″b), 1.69 (4H, complex, H-2′, -3′), 1.21 (3H, d, J=6.6 Hz, H-6″). ^{13}C-NMR (C_5D_5N) δ: 180.9 (C-2″), 176.7 (C-5″), 166.7 (C-9), 160.5 (C-4), 140.1 (C-7), 129.9 (C-2, -6), 127.0 (C-1), 119.4 (C-8), 116.7 (C-3, -5), 39.4 (C-1′ or -4′), 38.5 (C-1′ or -4′), 36.6 (C-4″), 35.0 (C-3″), 27.5 (C-2′ or -3′), 25.7 (C-2′ or -3′), 16.4 (C-6″).

Acetylation of 2 Compound **2** (10.0 mg) was treated with a mixture of Ac_2O and pyridine at room temperature for 6 h. The usual work-up followed by silica gel column chromatography with $CHCl_3$–acetone (9:1) yielded a monoacetate (**2a**) (8.9 mg) as a white amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3385 (NH), 3050, 2930, 2870 (CH), 1760 and 1700 (five-membered cyclic imide, intensity 1760<1700; absorption at 1700 cm^{-1} due to two carbonyl groups of imide and acetate), 1665 (secondary amide A band, C=O), 1620 (CH=CH), 1600, 1580, 1510 sh (aromatic ring), 1530 (secondary amide B band, NH), 1435, 1405, 1365, 1335, 1220, 1165, 1130, 1015, 915, 840. EI-MS *m/z* (%): 372.1721 (39) [M$^+$, Calcd for $C_{20}H_{24}N_2O_5$: 372.1686], 330.1606 (43) [(M−Ac)$^+$, Calcd for $C_{18}H_{22}N_2O_4$: 330.1581], 285 (6), 246 (8), 204 (35), 183 (100), 177 (26), 163 (29), 147 (98), 119 (38), 91 (30). ^{1}H-NMR ($CDCl_3$) δ: 7.59 (1H, d, J=15.9 Hz, H-7), 7.50 (2H, d, J=8.6 Hz, H-2, -6), 7.09 (2H, d, J=8.6 Hz, H-3, -5), 6.34 (1H, d, J=15.9 Hz, H-8), 5.91 (1H, br t, J=5.4 Hz, NH), 3.53 (2H, t, J=7.0 Hz, H-4′), 3.41 (2H, td, J=6.4, 5.4 Hz, H-1′), 2.92 (1H, dd, J=17.4, 9.0 Hz, H-4″a), 2.85 (1H, m, H-3″), 2.32 (1H, dd, J=17.4, 3.9 Hz, H-4″b), 2.30 (3H, s, Ac), 1.61 (4H, H-2′, -3′), 1.34 (3H, d, J=7.1 Hz, H-6″).

Hydrolysis of 2 Hydrolysis of **2** (20.0 mg) was performed by refluxing in 2 N NaOH for 4 h in an N_2 atmosphere. The reaction mixture, after being diluted with H_2O, was adjusted to pH 1 with 2 N HCl and extracted

with EtOAc. *p*-Coumaric acid and methylsuccinic acid (trace) were detected in the EtOAc layer by TLC ($CHCl_3$: MeOH : AcOH = 9 : 1 : 0.4); *p*-coumaric acid, *Rf* 0.60 (visualized by UV 254 nm and by spraying the plate with methylorange reagent); methylsuccinic acid, *Rf* 0.51 (visualized by spraying the plate with methylorange reagent). The H_2O layer, after being concentrated to a small volume, was added to 2 N NaOH to adjust to pH 12<. The H_2O residue had a characteristic odor, and putrescine was detected in it by TLC, *Rf* 0.08 (EtOH : 25% NH_3 = 4 : 1, visualized by spraying the plate with ninhydrin reagent followed by heating).

Acknowledgements We would like to thank Dr. Y. Shida and Miss Y. Kaneko for the measurement of the low- and high-resolution MS, and Mrs. C. Sakuma for the measurement of the COLOC spectrum. Thanks are also due to Dr. H. Taguchi (Tsumura Co.) for the measurement of the ORD spectrum.

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