Homoisoflavonoids from the Fibrous Roots of *Polygonatum odoratum* with Glucose Uptake-Stimulatory Activity in 3T3-L1 Adipocytes

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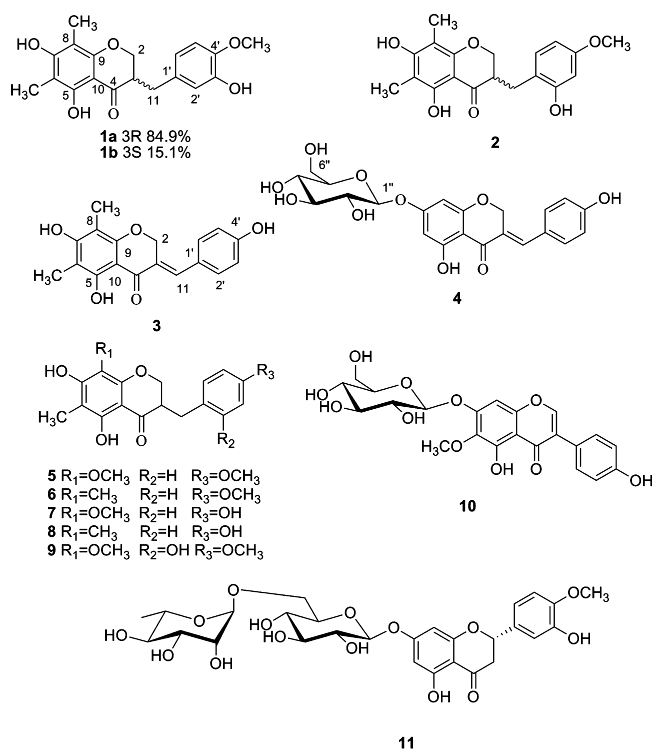
The EtOAc-soluble fraction of a 90% MeOH extract of the fibrous roots of *Polygonatum odoratum* was found to potentiate insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes. Bioassay-guided fractionation yielded nine homoisoflavonoids (**1**−**9**), four of which were new (**1**−**4**), together with an isoflavone glycoside (**10**) and a flavanone glycoside (**11**). The structures of new compounds were elucidated on the basis of extensive 1D and 2D NMR spectroscopy, and the absolute configurations were deduced by CD spectra. All 11 compounds showed effects of sensitizing adipocytes for insulin in a cell-based glucose uptake assay using 3T3-L1 adipocytes. The results indicate that homoisoflavonoids may be potential insulin sensitizers.

Polygonatum odoratum (Mill.) Druce, a famous Yin-nourishing herb in traditional Chinese medicine, has been used for hundreds of years to treat many diseases, especially diabetes.[1] Previous bioactivity investigation of *P. odoratum* showed that its MeOH extract had glucose-lowering effects on KK-Ay mice probably through increasing insulin sensitivity.[2] Our preliminary study also indicated that the EtOAc-soluble part of a 90% MeOH extract of the fibrous roots of *P. odoratum* potentiated insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes. Taken together, we speculated that *P. odoratum* might contain insulin-sensitizing secondary metabolites.

Previous phytochemical investigations on this species resulted in the isolation of steroidal saponins,[3–5] homoisoflavonoids,[6,7] a sterol,[5] etc. In this study, we investigated the chemical constituents of the 90% (v/v) MeOH extract of the fibrous roots of *P. odoratum* to seek the potential insulin sensitizers. Bioassay-directed fractionation led to the isolation of a series of homoisoflavonoids, including four new (**1**−**4**) and five known (**5**−**9**) analogues, along with an isoflavone glycoside (**10**) and a flavanone glycoside (**11**) from the active EtOAc-soluble fraction. In addition, their insulin-sensitizing effects were evaluated by a cell-based glucose uptake assay using differentiated 3T3-L1 adipocytes.



Results and Discussion

The EtOAc-soluble part of the 90% (v/v) MeOH extract of the fibrous roots of *P. odoratum* significantly increased insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes (Figure 1). This bioactive fraction was subjected to column chromatography on SP825 macroporous resins, silica gel, and Sephadex LH-20, and preparative TLC to yield 11 compounds (**1**−**11**), including four new homoisoflavonoids (**1**−**4**). The structures of compounds **1**−**4** were established by their spectroscopic data. The absolute configurations of compounds **1** and **2** were determined by CD experiments. The enantiomeric purity of compound **1** was measured by HPLC on a chiral column with UV/advanced laser polarimetric (ALP) detection. The seven known compounds were identified as 5,7-dihydroxy-6-methyl-8-methoxy-3-(4′-methoxybenzyl)chroman-4-one (**5**),[7] methylophiopogonanone B (**6**),[8] 5,7-dihydroxy-6-methyl-8-methoxy-3-(4′-hydroxybenzyl-)chroman-4-one (**7**),[6] 5,7-dihydroxy-6,8-dimethyl-3-(4′-hydroxybenzyl)chroman-4-one (**8**),[9] ophiopogonanone E (**9**),[10] tectoridin (**10**),[11] and hesperidin (**11**),[12] by comparing their spectroscopic data with the reported data. The absolute configurations of compounds **5**−**9** were also deduced by CD experiments. The CD spectra of compounds **5**−**8** exhibited negative Cotton effects around 295 nm, suggesting the *R* configuration at C-3.[13] Compound **9** was optically inactive, and the CD spectrum showed no significant Cotton effect, suggesting that it was racemic (Figure S35−39, Supporting Information). Compound **4** is the first reported glycoside having the 3-benzylidenechroman-4-one skeleton as the aglycone moiety. Compounds **9**−**11** were isolated from the genus *Polygonatum* for the first time. The ^{1}H and ^{13}C NMR spectroscopic data of the new homoisoflavonoids **1**−**4** are listed in Tables 1 and 2.

Compound **1** was obtained as colorless needles ($CHCl_3$−MeOH, 1:1). A molecular formula of $C_{19}H_{20}O_6$ was assigned on the basis of the HRESITOFMS giving an $[M - H]^-$ peak at *m/z* 343.1170 (calcd 343.1187). Its IR spectrum showed characteristic absorption bands for hydroxy (3415 cm^{-1}) and carbonyl (1635 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** exhibited the typical splitting pattern for a CH_2−CH−CH_2 moiety of a 3-benzyl-4-chromanone with the

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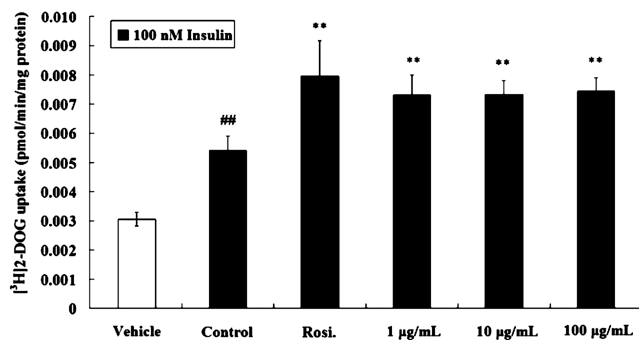

Figure 1. Effects of the EtOAc-soluble fraction on insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes. The glucose uptake effect of 1 μM rosiglitazone (Rosi.) with insulin (100 nM) stimulation served as a positive control (Rosi. vs control, $P < 0.05$). Data are the mean ± SD, $n = 3$. *$P < 0.05$, **$P < 0.01$ when compared with insulin-only group (100 nM); ##$P < 0.01$ when compared with vehicle.

H-2 signals occurring at δ 4.34 (1H, dd, $J = 4.6$, 11.4 Hz) and 4.15 (1H, dd, $J = 8.2$, 11.4 Hz), the H-3 signal occurring at δ 2.91(1H, m), and the H-11 signals each occurring as double doublets at δ 3.11 (1H, $J = 4.7$, 13.9 Hz) and 2.66 (1H, $J = 10.0$, 13.9 Hz), which were correlated with the carbons at δ 70.0 (C-2), 47.3 (C-3), and 32.6 (C-11) in the HSQC spectrum, respectively.[10,14] Comparison of the NMR data of **1** (Tables 1 and 2) with those of 5,7-dihydroxy-6,8-dimethyl-3-(3′-methoxy-4′-hydroxybenzyl)chroman-4-one showed their structural similarity, except for the different position of the B-ring methoxy group.[15] The location of the methoxy group at C-4′ was confirmed by the correlations of the methoxy protons at δ 3.83 to the oxygenated aromatic carbon at δ 147.4 and from this carbon to H-2′ (δ 6.80), H-5′ (δ 6.89), and H-6′ (δ 6.71) in the HMBC spectrum (Figure S40, Supporting Information). The observation of an NOE association between H-5′ (δ 6.89) and the methoxy protons (δ 3.83) in the NOESY spectrum further supported this conclusion. In the HMBC spectrum, the 5-OH (δ 12.48) proton showed $^3J_{C-H}$ correlations with the carbons at δ 104.3 (C-6) and 102.5 (C-10), and C-6 further showed long-range correlation to the methyl protons at δ 2.03, which suggested that the location of this methyl group was at C-6. Another methyl (δ 2.01) group was located at C-8 by the HMBC correlations from the methyl protons to C-7 (δ 163.2), C-8 (δ 103.2), and C-9 (δ 158.9). In addition, the CD spectrum of **1** exhibited a negative Cotton effect around 295 nm, which indicated the *R* configuration at C-3.[13] The ca. 85% enantiomeric purity of **1** was obtained from HPLC-UV/ALP coupling analysis on a chiral column (Figure S11, Supporting Information). Thus, the structure of **1** was elucidated as the mixture of 5,7-dihydroxy-6,8-dimethyl-3(*R*)-(3′-hydroxy-4′-methoxybenzyl)chroman-4-one (**1a**, 84.9%) and 5,7-dihydroxy-6,8-dimethyl-3(*S*)-(3′-hydroxy-4′-methoxybenzyl)chroman-4-one (**1b**, 15.1%).

Compound **2** was purified as colorless needles ($CHCl_3$−MeOH, 1:1). It had the same molecular formula ($C_{19}H_{20}O_6$) as **1** (*m/z* 343.1173 [M − H]$^-$ (calcd 343.1176) by HRESITOFMS. The IR and ^{1}H NMR spectra of **2** were similar to those of **1**, suggesting that compound **2** was also a homoisoflavanone with one methoxy group and two methyl groups. However, comparison of the NMR data of **2** with those of **1** suggested that **2** had a 1,2,4-trisubstituted B ring, compared to the 1,3,4-trisubstitued ring system of **1** (Tables 1 and 2). This conclusion was confirmed by the HMBC correlations between the methoxy protons at δ 3.72 (3H, s) and C-4′ (δ 161.7), as well as between C-4′ and protons at δ 6.38 (H-3′), 6.35 (H-5′), and 6.96 (H-6′), and the NOE interactions from δ 3.72 (3H, s) to δ 6.38 (H-3′) and 6.35 (H-5′) in the NOESY spectrum. Compound **2** was optically inactive, and the CD spectrum showed no significant Cotton effect. Hence, the structure of compound **2** was assigned as (±)-5,7-dihydroxy-6,8-dimethyl-3-(2′-hydroxy-4′-methoxybenzyl-)chroman-4-one.

Compound **3** was isolated as yellow needles (MeOH) and showed a pseudomolecular ion peak at *m/z* 311.0913 [M − H]$^-$ (calcd 311.0924) in the HRESITOFMS, corresponding to the molecular formula $C_{18}H_{16}O_5$. The IR spectrum exhibited the absorption peaks of hydroxy (3412 cm^{-1}) and carbonyl (1631 cm^{-1}) groups. The ^{1}H NMR showed the following representative signals: a methylene doublet at δ 5.34, an olefinic methine singlet at δ 7.67, two methyl singlets at δ 1.97 and 1.94, a hydrogen-bonded 5-OH singlet at δ 13.07, and an AA′BB′ spin system at δ 7.33 and 6.88, assigned to H-2′, 6′ and H-3′, 5′ (each 2H, d, $J = 8.6$ Hz). The ^{13}C NMR and DEPT spectra of **3** indicated 18 carbon signals, ascribed to two methyl, one methylene, five methine, and 10 quaternary carbons. These NMR data, especially signals at δ 5.34 (2H, d, $J = 1.6$) and

Table 1. ^{1}H NMR Data of Compounds **1**−**4**[a]

proton	**1**[b]	**2**[c]	**3**[d]	**4**[d]
2a	4.34, dd (4.6, 11.4)	4.24, dd (4.4, 11.3)	5.34, d (1.6)	5.39, brs
2b	4.15, dd (8.2, 11.4)	4.09, dd (7.6, 11.3)		
3	2.91, m	2.93, m		
6				6.16, d (2.0)
8				6.12, d (2.0)
11a	3.11, dd (4.7, 13.9)	3.16, dd (4.9, 13.6)	7.67, brs	7.71, brs
11b	2.66, dd (10.0, 13.9)	2.59, dd (9.8, 13.6)		
2′	6.80, d (2.1)		7.33, d (8.6)	7.34, d (8.5)
3′		6.38, d (2.5)	6.88, d (8.6)	6.89, d (8.5)
5′	6.89, d (8.2)	6.35, dd (2.5, 8.2)	6.88, d (8.6)	6.89, d (8.5)
6′	6.71, dd (2.1, 8.2)	6.96, d (8.2)	7.33, d (8.6)	7.34, d (8.5)
1″				4.97, d (7.5)
2″				3.21, m
3″				3.27, m
4″				3.12, m
5″				3.36, m
6″				3.44, m
5-OH	12.48, s		13.07, s	12.74, brs
7-OH			10.13, brs	
4′-OH			9.67, brs	10.22, brs
6-CH_3	2.03, s	1.98, s	1.97, s	
8-CH_3	2.01, s	1.96, s	1.94, s	
8-OCH_3				
4′-OCH_3	3.83, s	3.72, s		

[a] All compounds were measured at 500 MHz (δ in ppm). Proton coupling constants (*J*) in Hz are given in parentheses. [b] Measured in acetone-d_6. [c] Measured in methanol-d_4. [d] Measured in DMSO-d_6.

Table 2. ^{13}C NMR Data of Compounds **1**−**4**[a]

position	**1**[b]	**2**[c]	**3**[d]	**4**[d]
2	70.0	71.1	67.3	67.8
3	47.3	47.0	126.9	126.3
4	199.5	201.3	185.2	185.6
5	160.2	161.1	159.5[e]	164.4
6	104.3	105.2	103.9	97.3
7	163.2	164.3	162.8	165.8
8	103.2	104.2	102.7	95.8
9	158.9	159.8	156.7	162.2
10	102.5	103.3	102.1	103.7
11	32.6	28.7	136.6	138.0
1′	132.3	118.9	125.1	125.1
2′	116.8	158.1	130.0	133.5
3′	147.7	103.0	116.0	116.4
4′	147.4	161.7	159.7[e]	160.0
5′	112.9	106.2	116.0	116.4
6′	121.0	133.1	130.0	133.5
1″				100.1
2″				73.5
3″				76.7
4″				70.0
5″				77.5
6″				61.1
6-CH_3	8.0	8.3	8.2	
8-CH_3	7.6	7.9	7.8	
8-OCH_3				
4′-OCH_3	56.4	56.1		

[a] Data (δ in ppm) were measured at 125 MHz. [b] Measured in acetone-d_6. [c] Measured in methanol-d_4. [d] Measured in DMSO-d_6. [e] Data may be interchangeable.

δ 7.67 (1H, brs), which were correlated with the carbons at δ 67.3 (C-2) and 136.6 (C-11) in the HSQC spectrum, respectively, suggested a 3-benzylidenechroman-4-one skeleton for **3**.[16,17] The *E* geometry of the double bond at C-3 and C-11 was discerned by the characteristic chemical shift of H-11 at δ 7.67, as the corresponding signal for the *Z* isomer should resonate at a higher field (ca. δ 6.97).[18,19] The HMBC correlations between the 5-OH proton at δ 13.07 and C-5 (δ 159.5), C-6 (δ 103.9), and C-10 (δ 102.1) and between C-6 and the methyl protons at δ 1.97 indicated the presence of the methyl group at C-6. The position of a methyl at C-8 was confirmed by the long-range correlations from H-2 (δ 5.34) to C-9 (δ 156.7) and from the methyl protons (δ 1.94) to C-7 (δ 162.8), C-8 (δ 102.7), and C-9 (δ 156.7) in the HMBC spectrum. The structure of compound **3** was thus elucidated as (*E*)-5,7-dihydroxy-6,8-dimethyl-3-(4′-hydroxybenzylidene)chroman-4-one.

The HRESITOFMS of compound **4** showed an $[M - H]^-$ ion at *m/z* 445.1126 (calcd 445.1140), indicating the molecular formula $C_{22}H_{22}O_{10}$. A significant fragment ion in the ESI-MS^2 spectrum was observed at 283 $[M - H - 162]^-$, which was characteristic of the loss of one hexosyl unit. The IR spectrum exhibited absorption bands at 3425 (OH) and 1641 (C═O) and a broad band at 1150−1000 cm^{-1}, which further indicated its glycosidic nature.[20] The ^{1}H and ^{13}C NMR spectra of **4** also indicated a glycosidic structure. The ^{1}H NMR spectrum showed an anomeric proton at δ 4.97 (d, $J = 7.5$ H$_Z$), which was correlated with the anomeric carbon signal at δ 100.1 in the HSQC spectrum. Apart from the signals for the glucopyranosyl moiety, the ^{1}H NMR spectrum displayed signals of two *m*-coupled protons in ring A, one *p*-disubstituted ring B, and two hydroxy groups (Table 1). In addition, the ^{1}H NMR spectrum exhibited the same typical signals as **3** at δ 7.71 (brs) and 5.39 (brs) assigned to H-11 and H-2, indicating that it may also contain a 3-benzylidenechroman-4-one moiety. Furthermore, the ^{13}C NMR and DEPT spectra showed 12 aromatic carbons (including four oxgenated), one carbonyl carbon, one oxygenated methylene carbon, one olefinic methine carbon, and one olefinic quaternary carbon. These spectroscopic data (Tables 1 and 2) were essentially the same as those of 4′-demethylleucomin [5,7-dihydroxy-3-(4′-hydroxybenzylidene)chroman-4-one] except for the

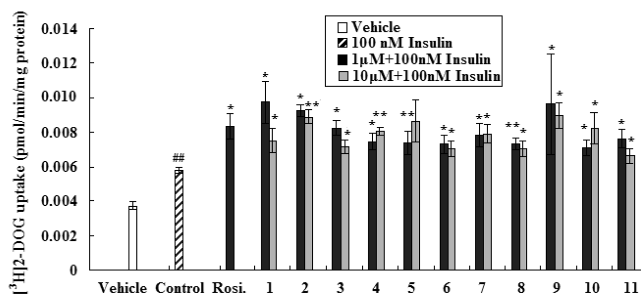

Figure 2. Effects of all compounds on insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes. The glucose uptake effect of 1 μM rosiglitazone (Rosi.) with insulin (100 nM) stimulation served as a positive control (Rosi. vs control, $P < 0.05$). Data are the mean ± SD, $n = 3$. $*P < 0.05$, $**P < 0.01$ when compared with insulin-only group (100 nM); $\#\#P < 0.01$ when compared with vehicle.

signals arising from the glucopyranosyl unit.[17] All the information mentioned above was indicative of **4** being a homoisoflavone glycoside. The sugar moiety was confirmed to be glucose by co-TLC with a standard sugar after hydrolysis, and the D-configuration was proved by GC results of the trimethylsilylthiazolidine derivative. The glycosidic position was revealed by the three-bond correlation in the HMBC spectrum between C-7 (δ 165.8) of the aglycone and the anomeric proton (δ 4.97) (Figure S41, Supporting Information). A large coupling constant for the anomeric proton in the ^{1}H NMR spectrum indicated the β configuration of the glucopyranosyl unit. Accordingly, compound **4** was elucidated as 4′-demethylleucomin 7-*O*-β-D-glucopyranoside or (*E*)-7-*O*-β-D-glucopyranoside-5-hydroxy-3-(4′-hydroxybenzylidene)chroman-4-one.

Cellular glucose uptake is the major rate-limiting step in the regulation of glucose metabolism by insulin, and defects in its regulation lead to insulin resistance.[21] Facilitating insulin-mediated glucose uptake of skeletal muscle and adipose tissue is one of the pivotal hallmarks of insulin sensitization.[22] Therefore, we examined the effects of all isolated compounds on insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes.

As shown in Figure 2, all 11 compounds showed the effects of improving insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes compared with the treatment of 100 nM insulin. Especially, compound **1** exhibited the most potent glucose uptake-stimulatory activity at the concentration of 1 μM. Preliminary structure−activity relationship analysis indicated that compounds with an additional hydroxy group at C-2′ or C-3′ (compounds **1**, **2**, and **9**) showed much stronger activities than compounds with only one hydroxy or methoxy group at C-4′ (compounds **5**−**8**). In summary, the series of natural homoisoflavonoids increased insulin-stimulated glucose uptake of 3T3-L1 cells (Figure 2), which has not been reported previously. These results indicate that homoisoflavonoids are able to ameliorate insulin resistance peripherally and can be potentially developed as insulin sensitizers for the treatment of diabetes. However, further in vivo investigations are necessary.

Experimental Section

General Experimental Procedures. Melting points were determined on an X-4 micro melting point apparatus without correction. Optical rotations were obtained using a JASCO P-1020 digital polarimeter. UV spectra were recorded in MeOH on a Shimadzu UV-2501 PC spectrophotometer. CD spectra were recorded on a JASCO J-810 spectropolarimeter. Online HPLC-UV/ALP was performed on a Shimadzu LC 20 instrument with UV SPD-20A detector coupled with an advanced laser polarimeter detector (PDR-Chiral Inc., USA), and a Chiralpak IC chiral column (250 × 4.6 mm, 5 μm, Daicel Chiral Technologies Co., Ltd.) was employed. IR spectra were measured on a Shimadzu FTIR-8400s spectrophotometer. NMR spectra were re-

corded on a Bruker AV-500 spectrometer with TMS as the internal standard. ESIMS and HRESITOFMS experiments were performed on an Agilent 1100 Series MSD Trap mass spectrometer and an Agilent 6210 ESITOF spectrometer, respectively. TLC was performed on precoated silica gel GF254 plates (Qingdao Haiyang Chemical Co. Ltd.). Column chromatography was carried out with silica gel H (Qingdao Haiyang Chemical Co. Ltd.), Sephadex LH-20 (Pharmacia, Sweden), and macroporous resin SP825 (Mitsubishi Chemical, Japan). L-Cysteine methyl ester hydrochloride, trimethylchlorosilane, hexamethyldisilazane, and standard D-glucose were purchased from Sigma-Aldrich (Shanghai) Trading Co. Ltd.

Plant Material. Dried fibrous roots of *Polygonatum odoratum* were collected in Shaodong County, Hunan Province, People's Republic of China, in October 2007, and identified by one of the authors (B.-Y.Y.). A voucher specimen (Herbarium No. 20071010) of the plant is deposited at the herbarium of the Department of Complex Prescription of TCM, China Pharmaceutical University.

Extraction and Isolation. Air-dried fibrous roots of the plant (15 kg) were refluxed three times with 90% (v/v) MeOH. The combined extracts were evaporated to dryness under vacuum, and the residue was suspended in H_2O and extracted with EtOAc. The EtOAc-soluble fraction (680 g) was chromatographed on a silica gel column using a gradient of $CHCl_3$−MeOH−H_2O (100:0:0 to 50:50:5, v/v/v) and yielded 14 fractions (A−N) based on the TLC profiles.

Fraction B was subjected to CC on silica gel, eluted with a gradient of petroleum ether−EtOAc (95:5 to 50:50, v/v), to yield five subfractions (B1−B5). Fraction B2 was separated by a silica gel column eluted with petroleum ether−EtOAc (8:2) to obtain B2-1. Fraction B2-1 was purified with Sephadex LH-20 eluted with $CHCl_3$−MeOH (1:1) to obtain compound **6** (13 mg). Fraction B3 was subjected to CC on silica gel eluted with petroleum ether−Me_2CO (8.5:1.5) to yield compound **5** (18 mg). Fraction B4 was chromatographed on a silica gel column eluted with petroleum ether−EtOAc (9:1 to 7:3) to obtain five fractions (B4a to B4e). Fraction B4b was subjected to CC on Sephadex LH-20 eluted with $CHCl_3$−MeOH (1:1) to give three subfractions (B4ba to B4bc). Compound **1** (10 mg) was crystallized from B4bb in $CHCl_3$−MeOH (1:1). Compounds **2** (38 mg) and **8** (280 mg) were isolated from the remaining part of B4bb by a silica gel column eluted with $CHCl_3$−MeOH (95:5). Fraction B4bc was purified by Sephadex LH-20 with MeOH as mobile phase to yield compound **3** (3 mg). Compounds **7** (8 mg) and **9** (13 mg) were obtained from fraction B5 by normal-phase preparative TLC developed with $CHCl_3$−MeOH (95:5).

Fractions G and L were subjected to macroporous resin SP-825 CC eluted successively with EtOH−H_2O (0:100, 30:70, 50:50, 70:30, 95:5, v/v) to give five subfractions (G1−G5, L1−L5), respectively. Subfraction G3 was chromatographed on Sephadex LH-20 with MeOH−H_2O (8:2) as mobile phase to afford G3a and G3b. Fraction G3a was further purified by Sephadex LH-20, eluted with MeOH, to obtain compound **4** (8 mg). Fraction G3b was passed through Sephadex LH-20, using $CHCl_3$−MeOH−H_2O (2:2.7:1) as mobile phase, to yield G3ba, from which compound **10** (6 mg) was obtained. Subfraction L3 was subjected to Sephadex LH-20 with MeOH as mobile phase to afford L3a−L3d. Fraction L3c was chromatographed on a silica gel column eluted with $CHCl_3$−MeOH−H_2O (8:2:0.2) to obtain compound **11** (15 mg).

The enantiomeric purity of compound **1** was measured by online HPLC-UV/ALP coupling analysis on a Chiralpak IC column (250 × 4.6 mm, 5 μm, Daicel Chiral Technologies Co., Ltd.) at 35 °C, eluting with *n*-hexane−2-propanol−HOAc (80:20:0.1, v/v/v) at a flow rate of 1.0 mL/min. The chromatographic profile was detected at 220 nm, and area percentages of peak 1 (t_R = 9.1 min) and peak 2 (t_R = 10.8 min) were determined to be 84.9% and 15.1%, respectively.

5,7-Dihydroxy-6,8-dimethyl-3(*R*)-(3′-hydroxy-4′-methoxybenzyl)-chroman-4-one (1a, 84.9%) and 5,7-dihydroxy-6,8-dimethyl-3(*S*)-(3′-hydroxy-4′-methoxybenzyl)chroman-4-one (1b, 15.1%): colorless needles ($CHCl_3$−MeOH, 1:1); mp 229−230 °C; $[\alpha]^{20}_D$ −44.9 (*c* 0.15, acetone); UV (MeOH) λ_{max} (log ε) 204 (4.67), 295 (4.34), 344 (3.58) nm; CD (*c* 5.29 × 10^{-5} M, MeOH) λ_{max} nm ($\Delta\varepsilon$) 297 (− 1.86); IR (KBr) ν_{max} 3421 (OH), 2924, 1639, 1611, 1516, 1472, 1439, 1388, 1242, 1168, 1111, 1080, 1027, 959, 798 cm^{-1}; ^1H NMR (acetone-d_6, 500 MH_Z) data, see Table 1; ^{13}C NMR (acetone-d_6, 125 MH_Z) data, see Table 2; ESIMS *m/z* 343 [M − H]$^-$; HRESITOFMS *m/z* 343.1170 [M − H]$^-$ (calcd for $C_{19}H_{19}O_6$, 343.1187).

(±)-5,7-Dihydroxy-6,8-dimethyl-3-(2′-hydroxy-4′-methoxybenzyl)-chroman-4-one (2): colorless needles ($CHCl_3$−CH_3OH, 1:1); mp 168−169 °C; UV (MeOH) λ_{max} (log ε) 206 (4.60), 295 (4.28), 343 (3.50) nm; IR (KBr) ν_{max} 3375 (OH), 2919, 1622, 1525, 1469, 1385, 1366, 1270, 1245, 1162, 1129, 1108, 1070, 1037, 955, 832, 791 cm^{-1}; ^1H NMR (methanol-d_4, 500 MH_Z) data, see Table 1; ^{13}C NMR (methanol-d_4, 125 MH_Z) data, see Table 2; ESIMS *m/z* 343 [M − H]$^-$; HRESITOFMS *m/z* 343.1173 [M − H]$^-$ (calcd for $C_{19}H_{19}O_6$, 343.1176).

(*E*)-5,7-Dihydroxy-6,8-dimethyl-3-(4′-hydroxybenzylidene)chroman-4-one (3): yellow needles (MeOH); mp 217−218 °C; UV (MeOH) λ_{max} (log ε) 207 (4.21), 364 (4.16) nm; IR (KBr) ν_{max} 3412 (OH), 2922, 1631 (C=O), 1607, 1550, 1513, 1469, 1370, 1327, 1198, 1175, 1155, 826 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MH_Z) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MH_Z) data, see Table 2; ESIMS *m/z* 311 [M − H]$^-$; HRESITOFMS *m/z* 311.0913 [M − H]$^-$ (calcd for $C_{18}H_{15}O_5$, 311.0924).

4′-Demethylleucomin 7-*O*-*β*-D-glucopyranoside (4): yellow needles (MeOH); mp 252−253 °C; $[\alpha]^{20}_D$ −67.4 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.52), 360 (4.40) nm; IR (KBr) ν_{max} 3425 (OH), 2926, 1641, 1587, 1514, 1444, 1398, 1365, 1291, 1150−1000, 820 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MH_Z) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MH_Z) data, see Table 2; ESIMS *m/z* 445 [M − H]$^-$, 283 [M − H]$^-$; HRESITOFMS *m/z* 445.1126 [M − H]$^-$ (calcd for $C_{22}H_{21}O_{10}$, 445.1140).

Acid Hydrolysis of 4. Compound **4** (2 mg) was refluxed with 2 mL of 2 M HCl (dioxane−H_2O, 1:1) at 100 °C for 4 h. After the dioxane was removed, the solution was diluted with H_2O and extracted with EtOAc (1 mL × 3). The aqueous layer was evaporated under vacuum, diluted repeatedly with H_2O, evaporated under vacuum to obtain a neutral residue, and then analyzed by TLC over silica gel (Me_2CO−*n*-BuOH−H_2O, 6:3:1) together with authentic sugar sample (glucose, R_f = 0.49). The remaining residue was dissolved in pyridine (150 μL), to which 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 1.5 h; then 150 μL of HMDS−TMCS (hexamethyldisilazane−trimethylchlorosilane, 2:1) was added, and the mixture was stirred at 60 °C for another 30 min.[23] After centrifugation, the supernatant was analyzed by GC under the following conditions: capillary column, CP-SIL 5CB (0.32 mm × 30 m); detection, FID; detector temperature, 280 °C; injection temperature, 250 °C; initial temperature, 140 °C, then raised to 280 at 10 °C/min; carrier, N_2 gas. The absolute configuration of the monosaccharide was confirmed to be D-glucose by comparison of the retention time (t_R) of the monosaccharide derivative with that of a standard sample derivative in a similar way (D-glucose derivative t_R = 10.75 min).

3T3-L1 Cell Culture and Insulin-Stimulated Glucose Uptake.[24] 3T3-L1 preadipocytes were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% calf serum (CS) at 37 °C in a 5% CO_2 incubator until confluent and maintained for an additional 2 days. For cell differentiation of glucose uptake, cells were seeded in DMEM with 10% newborn bovine serum (NBS, GIBCO) at a density of 4 × 10^4 cells/mL in 24-well culture plates and induced to differentiate with 10% fetal calf serum (FCS, Gibco) DMEM supplemented with 10 μg/mL insulin, 0.5 μM dexamethasone, and 0.5 mM isobutylmethylxanthine (IBMX) for 48 h. Then, the medium was replaced with 20% FCS/DMEM containing 10 μg/mL insulin. After another 2 days, the medium was subsequently replaced with 20% FCS/DMEM, which was changed every 2 days thereafter until the cells were fully differentiated. Over 95% of the preadipocytes differentiated into adipocytes by day 10, as determined by Oil Red O stain; the tested compounds were added for an additional 3 days. Since the compounds were solubilized in DMSO, control cells were treated with matching concentrations of DMSO, and the final concentration of DMSO was kept below 0.1%. At day 12 of differentiation, the cells were rinsed with serum-free DMEM and incubated for 2 h in serum-free DMEM. The cells were washed twice with freshly prepared Krebs-Ringer-Phosphate-HEPES (KRPH) buffer (136 mM NaCl, 20 mM HEPES (pH 7.4), 5 mM sodium phosphate buffer (NaH_2PO_4−Na_2HPO_4·$7H_2O$, pH 7.4), 4.7 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$). After washing, the cells were incubated in KRB buffer without insulin (the negative control group) or with 100 nM insulin (the tested compounds) for 30 min. Then the buffer was replaced with 10 μCi/mL of [^{3}H]2-deoxy-D-glucose in KRPH buffer supplemented with 100 μM 2-deoxy-D-glucose and incubated at 37 °C for 10 min. The cells were rinsed with ice-cold PBS three times to terminate the reaction. Plates were drained briefly, and cells were lysed with 125 μL/well 0.2 N NaOH and mixed by pipetting. Lysate (100 μL) was

added to a scintillation vial, and 1 mL of scintillation fluid was added. The vials were mixed and counted. The rest of the lysate was used to determine the protein concentration using the BCA protein assay with bovine serum albumen as standard. The glucose uptake values are expressed as "pmol radioactive 2-deoxyglucose taken up per minute and per mg protein".

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Supporting Information Available: NMR and HRESITOFMS spectra of compounds **1**−**4**. CD spectra of compounds **1**, **2**, and **5**−**9**. HPLC-UV/ALP chromatogram of **1**. Key correlations observed in the HMBC and NOESY NMR spectra of **1**. Key correlations observed in the HMBC NMR spectrum of **4**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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