Inhibitors of Aldose Reductase and Formation of Advanced Glycation End-Products in Moutan Cortex (*Paeonia suffruticosa*)

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The methanol extract of Moutan cortex (*Paeonia suffruticosa*) afforded two new compounds, 8-*O*-benzoylpaeonidanin (**1**) and 5-hydroxy-3*S*-hydroxymethyl-6-methyl-2,3-dihydrobenzofuran (**2**), in addition to 4-*O*-butylpaeoniflorin (**3**) as an artifact of the separation, seven monoterpene glycosides (**4**−**10**), two monoterpenes (**11**, **12**), four acetophenones (**13**−**16**), and two triterpenes (**17**, **18**). The structures of the compounds were determined by spectroscopic methods, and the compounds were evaluated for inhibitory effects against rat lens aldose reductase (RLAR) and advanced glycation end-product (AGEs) formation. Compounds **17** and **18** showed the most potent inhibitory activity against RLAR, with IC_{50} values of 11.4 and 28.8 μM, respectively. Compounds **3** and **6** also inhibited RLAR with IC_{50} values of 36.2 and 44.6 μM, respectively. The positive control, 3,3-tetramethyleneglutamic acid, had an IC_{50} value of 31.8 μM. Compounds **3** and **6** inhibited AGE formation with IC_{50} values of 10.8 and 11.3 μM, respectively. Compound **2** had an IC_{50} value of 177.0 μM, whereas the positive control, aminoguanidine, had an IC_{50} value of 1026.8 μM.

The Diabetes Control and Complication Trial (DCCT) showed that hyperglycemia is a key risk factor for the development of diabetic complications.[1,2] Diabetic complications can arise from increased flux through the polyol pathway and the formation of advanced glycation end-products (AGE).[3] Aldose reductase (AR) (alditol: NAD $(P)^+$ 1-oxidoreductase, EC 1.1.1.21) reduces the aldehyde form of glucose to sorbitol in the presence of NADPH as a cofactor. Sorbitol dehydrogenase oxidizes sorbitol to fructose in the polyol pathway, leading to loss of the functional integrity of the lens and subsequent cataract formation.[4] Formation of AGEs relates to the development of cataracts, diabetic complications, uremia, Alzheimer's disease, and other disorders.[5] Therefore, inhibiting AR and AGE formation may have the potential to treat long-term diabetic complications.[6]

Moutan cortex ("Mok-Dan-Pi" in Korean), the root bark of *Paeonia suffruticosa* Andrew (Paeoniaceae), is an analgesic, sedative, and anti-inflammatory agent that has been used as a remedy for cardiovascular disorders, extravagated blood, and female genital diseases.[7] Numerous monoterpene glycosides and acetophenones have been isolated from this plant.[7,11,15,16,18] Moutan cortex is also found in antidiabetic traditional Chinese medicine formulas such as Palmi-Jihuang-hwan (Hachimi-jio-gan, Kampo medicine).[8] However, the effects on antidiabetic complications and identity of the bioactive compounds remain unclear.[9,10] We have identified the bioactive compounds in Moutan cortex and evaluated their ability to inhibit RLAR and AGE formation.

Results and Discussion

Eighteen compounds were obtained from the MeOH extract of Moutan cortex (**1**−**18**) (Figure 1). From this extract, the identities of 15 known compounds were verified by comparing their physicochemical and spectroscopic data to published values for paeoniflorin,[12] benzoylpaeoniflorin,[13] galloylpaeoniflorin,[14] oxypaeoniflorin,[12] mudanpinoside H,[15] α- and β-benzoyloxypaeoniflorin,[16] paeoniflorigenone,[14] 6-methoxypaeoniflorigenone,[12] paeonol,[17] paeonolide,[18] paeonoside,[19] apiopaeonoside,[18] palbinone,[20] and 30-norhederagenin.[21]

Compound **1** was isolated as an amorphous, white powder. Its IR spectrum displayed absorption bands arising from hydroxy (3419 cm^{-1}) and ester carbonyl (1716 cm^{-1}) groups. The UV absorption showed peaks with λ_{max} at 241, 250, and 284 nm, indicating a benzoyl chromophore. The HRFABMS spectrum of **1** revealed an $[M - H_3O]^-$ ion (*m*/*z* 579.5599), corresponding to the molecular formula $C_{31}H_{34}O_{12}$ (the calculated *m*/*z* was 579.5499) and indicating 15 degrees of unsaturation. The ^1H and ^{13}C NMR spectra of **1** were closely related to those of paeonidanin,[22,23] except for signals arising from a monosubstituted aromatic ring and an ester carbonyl group that suggested the presence of an additional benzoyl group. The ^1H and ^{13}C NMR data of **1** (Table 1) showed the presence of aromatic proton signals of two benzoyl moieties [δ_H 7.42 (2H, dd, J = 7.5, 1.6 Hz), 7.52 (1H, dd, J = 7.5, 1.6 Hz), 7.62 (1H, dd, J = 7.8, 1.2 Hz), 7.48 (2H, d, J = 7.8 Hz), 7.99 (2H, d, J = 7.5 Hz), 8.00 (2H, d, J = 7.8 Hz, and [δ_C 129.7 (C-3‴, 5‴), 129.8 (C-3″, 5″), 130.7 (C-2‴, 6‴), 130.8 (C-2″, 6″), 131.3 (C-1′), 131.5 (C-1‴), 134.6 (C-4‴), 134.7 (C-4′)] and two ester carbonyl signals [δ_C 167.8 (C-7″), 168.0 (C-7‴)]. The sugar moiety in **1** was deduced to be a β-D-glucopyranosyl unit because of the anomeric carbon and proton signals at δ_C 100.0 (C-1′) and δ_H 4.60 (1H, d, J = 7.8 Hz, H-1′) and the results of acid hydrolysis. The HMBC (Figure 2) spectra showed a correlation between H-1′ (δ_H 4.60) and C-1 (δ_C 88.7), suggesting that a sugar moiety was correlated with a monoterpene unit at C-1; a peak between H-8 (δ_H 4.76) and C-7‴ (δ_C 168.0) indicating that a benzoyl moiety was correlated with a monoterpene unit at C-8; and correlation of H-6′ (δ_H 4.53, 4.65) with C-7″ (δ_C 167.8) indicating a connection between paeonidanin and a benzoyl moiety at C-6′. The presence of a methoxy group at C-9 of **1** was identified by HMBC correlations of the methoxy protons (δ_H 5.05) to C-5 (δ_C 49.4), C-7 (δ_C 64.9), and C-9 (δ_C 107.7) (Figure 2). Further confirmation of a linkage between the 6′-benzoyl-*O*-β-D-glucopyranosyl moiety and C-1 was given by

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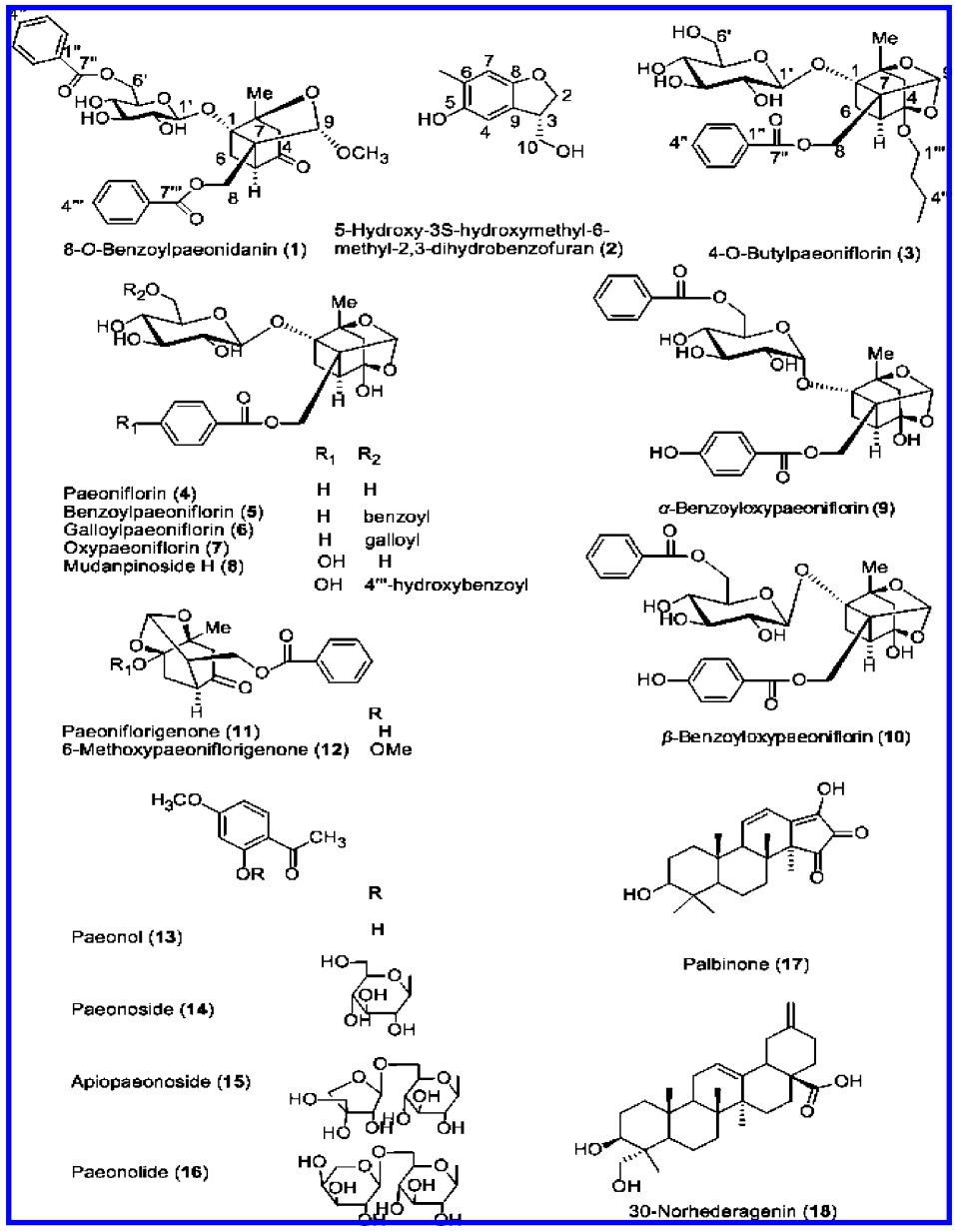

Figure 1. Chemical structures of isolates (**1**−**18**) from Moutan cortex.

proton correlations in the ROESY spectrum between H-1′/H-6α, H-1′/H-8α, and H-1′/10-CH_3 (Figure 3). The configurations of the stereogenic centers in the monoterpene unit were initially assigned by comparison to the spectra of related compounds and was confirmed by the ROESY spectrum.[22] The ROESY spectra also showed cross-correlations between the pairs of protons H-1′/H-6α, H-6α/H-8α, H-6α/H-5, H-8α/H-5, and H-8β/H-9, indicating an α-orientation of the 9-OMe (Figure 3).[23] With these assignments, **1** was identified as 8-*O*-benzoylpaeonidanin.

Compound **2** was obtained as a white powder. The presence of hydroxy groups was supported by peaks in the IR spectrum at 3364 and 3164 cm^{-1}. The UV absorption showed the presence of a benzene ring as determined by peaks with λ_{max} at 219 and 250 nm. The positive ion mode HRESIMS of compound **2** exhibited a molecular ion peak for $[M]^+$ at an *m*/*z* of 180.0768, which was consistent with a molecular formula of $C_{10}H_{12}O_3$ (the calculated *m*/*z* was 180.0786) and five degrees of unsaturation. The mass spectrum peaks for $[M - 31]^+$ at an *m*/*z* of 149 and $[M - H-58]^+$ at an *m*/*z* of 121 strongly suggested the presence of a propanoyl unit. The fragment ion peaks at *m*/*z* of 91 $[C_7H_7]^-$, 77 $[C_6H_5]^-$, and 57 $[M - H - 122]^+$ indicated the presence of a 5-hydroxy-6-methyldihydrobenzofuran unit (*m*/*z* 122). The ^1H NMR spectrum showed a methyl signal at δ_H 2.11 (3H, s, CH_3-11); two methylene signals at δ_H [3.54 (1H, dd, J = 10.2, 7.8 Hz, H-10a) and 3.69 (1H, dd, J = 10.2, 5.4 Hz, H-10b)] and at δ_H [4.33 (1H, dd, J = 8.7, 5.2 Hz, H-2a) and 4.51 (1H, dd, J = 9.0, 8.7 Hz, H-2b)]; and one methine signal at δ_H 3.48 (1H, m, H-3).

The ^{13}C NMR and DEPT spectra displayed 10 carbon signals, which indicated four quaternary, one methine, two methylene, and one methyl carbon. The ^1H NMR spectrum showed singlet aromatic protons [δ_H 6.66 (1H, s, H-4) and 6.46 (1H, s, H-7)]. The locations of the methyl and hydroxymethylene groups were assigned by correlations revealed in the HMBC experiment (Figure 2) between the 2-hydroxymethylene proton (δ_H 3.48) and C-2 (δ_C 75.4), C-3 (δ_C 46.7), and C-9 (δ_C 126.8), as well as from 11-CH_3 (δ_H 2.11) to C-5 (δ_C 150.5), C-6 (δ_C 125.7), and C-7 (δ_C 111.9). The 3*S* absolute configuration of **2** was confirmed by comparing the sign of the specific rotation {$[\alpha]^{25}_D$ +6.7 (*c* 0.16, MeOH)} with 3(*S*)-methyl-2,3-dihydrobenzofuran {$[\alpha]^{23}_D$ +74 (*c* 0.021, CH_2Cl_2)} and 3(*S*)-methyl-2,3-dihydrobenzofuran-7-carboxylic acid {$[\alpha]^{23}_D$ +38 (*c* 0.040, CH_2Cl_2)}.[24] From these data, compound **2** was identified as 5-hydroxy-3*S*-hydroxymethyl-6-methyl-2,3-dihydrobenzofuran.[25]

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **3** in Methanol-d_4

	1				**3**	
position	^{1}H[a]	^{13}C[b]	HMBC	ROESY	^{1}H[a]	^{13}C[b]
1		88.7	10-CH_3, H-3, H-6, H-1′			89.4
2		87.5	10-CH_3, H-3, H-6			87.4
3a	2.37 (1H, d, 18.3)	50.0	10-CH_3, H-5	H3a/H3b, 10-CH_3	1.93 (1H, d, 12.2)	42.9
3b	2.50 (1H, d, 18.3)				2.15 (1H, d, 12.2)	
4		209.1	H-3, H-5, H-6			109.5
5	2.89 (1H, dd, 7.6, 10.8)	49.4	H-3, H-6, H-8, H-9	H5/6b, H8a, H8b, H1′	2.77 (1H, dd, 6.6, 1.2)	41.6
6a	1.88 (1H, d, 10.8)	27.5	H-5	H6a/H6b H6b/H1′, H8a	1.91 (1H, d, 11.2)	23.6
6b	2.90 (1H, dd, 10.8)				2.49 (1H, dd, 11.2, 7.0)	
7		64.9	H-5, H-6, H-8, H-9	H8a/H8b		71.9
8a	4.67 (1H, d, 11.8)	63.9	H-6, H-9	H8a/H5, H6b	4.76 (2H, s)	63.1
8b	4.79 (1H, d, 11.8)					
9	5.05 (1H, s)	107.7	9-OMe, H-8	H9/H8b, 9-OCH_3	5.44 (1H, s)	102.7
1′	4.60 (1H, d, 7.8)	100.0	H-2′, H-3′	H1′/H6b, H5′	4.53 (1H, d, 7.5)	100.4
2′	3.33 (1H, m)[c]	75.1	H-1′, H-3′		3.25 (1H, dd, 7.5, 9.3)	78.2
3′	3.42 (1H, t, 8.7)	78.1	H-2′, H-4′	H2′/H6′b	3.42 (1H, m)	78.3
4′	3.37 (1H, m)	72.2	H-3′, H-5′	H4′a/H6′a, 6′b	3.55 (1H, m)	72.0
5′	3.63 (1H, dd, 9.3, 7.0)	75.4	H-3′, H-4′	H5′/H1′, H6′b	3.61 (1H, m)	75.2
6′a	4.53 (1H, dd, 11.7, 7.0)	65.2	H-5′, H-4′	H6′a/5′	3.64 (1H, dd, 11.7, 6.3)	61.9
6′b	4.65 (1H, dd, 11.7, 2.7)			H6′b/H2′, H5′	3.85 (1H, d, 11.7)	
1″		131.3	H-3″, 5″			131.4
2″	8.00 (1H, d, 7.8)	130.8	H-3″, 4″, 6″	H2″/H3″	8.05 (1H, d, 8.0)	130.8
3″	7.48 (1H, d, 7.8)	129.8	H-2″, 4″, 5″	H3″/H2″	7.49 (1H, d, 8.0)	129.8
4″	7.62 (1H, dd, 7.8, 1.2)	134.7	H-2″, 3″, 4″, 6″	H4″/H3″, H5″	7.62 (1H, d, 8.0)	134.6
5″	7.48 (1H, d, 7.8)	129.8	H-3″, 4″, 6″	H5″/H6″	7.49 (1H, d, 8.0)	129.8
6″	8.00 (1H, d, 7.8)	130.8	H-2″, 4″, 5″	H6″/H5″	8.05 (1H, d, 8.0)	130.8
7″		167.8	H-6′, H-2″, 6″			167.1
1‴		131.5	H-3‴, 5‴		3.25 (2H, m)	64.5
2‴	7.99 (1H, d, 7.5)	130.7	H-3‴, 4‴, 6‴	H2‴/H3‴	1.53 (2H, q, 6.5)	33.2
3‴	7.42 (1H, dd, 7.5, 1.6)	129.7	H-2‴, 4‴, 5‴	H3‴/H2‴	1.32 (2H, m)	20.3
4‴	7.52 (1H, dd, 7.5, 1.6)	134.6	H-2‴, 3‴, 4‴, 6‴	H4‴/H3‴, H5‴	0.90 (3H, t, 7.1)	14.3
5‴	7.42 (1H, dd, 7.5, 1.6)	129.7	H-3‴, 4‴, 6‴	H5‴/H6‴		
6‴	7.99 (1H, d, 7.5)	130.7	H-2‴, 4‴, 5‴	H6‴/H5‴		
7‴		168.0	H-8, H-2‴, 6‴			
−OCH_3	3.25 (3H, s)	55.9	H-9	9-OCH_3/H8b, H9,		
10-CH_3	1.31 (3H, s)	20.8	H-3	10-CH_3/H3a, H3b, H1′	1.38 (3H, s)	19.8

[a] δ_H ppm (mult., J in Hz), 300 MHz. [b] δ_C ppm, 75 MHz. [c] Overlapped with solvent signals.

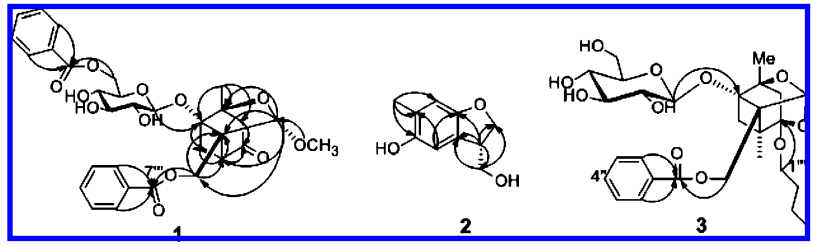

Figure 2. Selected HMBC correlations of **1**−**3**.

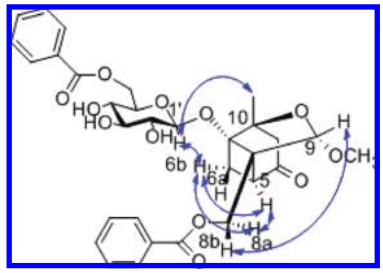

Figure 3. Selected ROESY correlations of **1**.

Compound **3** was isolated as an optically active white foam. The IR spectrum showed absorption bands for hydroxy (3396 and 2932 cm^{-1}) and carbonyl (1712 cm^{-1}) groups and a glycosidic moiety (955 cm^{-1}). The UV absorption showed peaks with λ_{max} at 210, 233, 240, and 250 nm, indicating a typical benzoylic chromophore. The positive ion mode HRESIMS of **3** exhibited a molecular ion peak for $[M]^+$ at an m/z of 536.2234, which was consistent with the molecular formula $C_{27}H_{36}O_{11}$ (the calculated m/z was 536.2258) and 10 degrees of unsaturation. In the EIMS spectrum, the $[M - H]^-$ ion readily eliminated a glucosyl group to form fragmentation peaks with m/z ratios of 179 and 356 (for the $[M - 179 - H]^+$ ion). A noteworthy feature was the moderate abundance of the $[M - 86]^+$ ion at m/z 450, which was attributed to the loss of HCHO (formaldehyde) and C_4H_8, from a butyl moiety connected to the monoterpene unit at C-4. In addition, the presence of a fragmentation ion peak at m/z of 416, $[M - 121 + H]^+$, corresponded to the loss of a benzoyloxy moiety ($C_7H_5O_2$). The ^{1}H and ^{13}C NMR spectra of **3** (summarized in Table 1) closely resembled the data for paeoniflorin,[26,27] apart from the appearance of signals arising from a butyl moiety [δ_H 3.56 (2H, m), δ_C 64.5; δ_H 1.53 (2H, q), δ_C 33.2; δ_H 1.32 (2H,m), δ_C 20.2; δ_H 0.90 (3H,m), δ_C 14.3].[26] The sugar moiety linkage was ultimately attributed to β-D-glucose from the coupling constant of one anomeric proton [δ_H 4.53 (1H, d, J = 7.5 Hz, H-1′)] and by comparing its retention time to those of standard sugars. The linkage of a butyl group to C-4 was assigned by an HMBC correlation between H-1‴ (δ_H 3.25) and C-4 (δ_C 109.5). On the basis of these observations, the structure of **3** was revealed to be 4-*O*-butylpaeoniflorin (Figure 2).

Table 2. Inhibitory Effects of Moutan Cortex Extracts on Rat Lens Aldose Reductase (RLAR) and the Formation of Advanced Glycation End-Products (AGEs) *in Vitro*

	IC_{50} (μg/mL)[a]					
	main extract	subsequent partitions			positive control	
assay	MeOH	*n*-hexane	EtOAc	*n*-BuOH	TMG	AG
RLAR	10.3 ± 0.70	5.7 ± 2.33	3.2 ± 0.15	6.3 ± 2.48	5.3 ± 0.31	
AGEs	5.9 ± 0.11	35.1 ± 1.02	1.8 ± 0.08	3.5 ± 0.29		76.0 ± 1.08

[a] IC_{50} values were calculated from dose-dependent inhibition curves; 3,3-tetramethyleneglutamic acid (TMG); aminoguanidine (AG). Inhibitory effects are displayed as mean ± SE of triplicate experiments.

Table 3. Inhibitory Effects of Compounds **1**−**18** from Moutan Cortex on Rat Lens Aldose Reductase (RLAR) and the Formation of Advanced Glycation End-Products (AGEs) *in Vitro*

	IC_{50}(μM)[a]	
compound	RLAR	AGEs
8-*O*-benzoylpaeonidanin (**1**)	>50	>500
5-hydroxy-3*S*-hydroxymethyl-6-methyl-2,3-dihydrobenzofuran (**2**)	>50	177.0 ± 14.95
4-*O*-butylpaeoniflorin (**3**)	36.2 ± 4.47	10.8 ± 0.63
paeoniflorin (**4**)	>50	>500
benzoylpaeoniflorin (**5**)	>50	>500
galloylpaeoniflorin (**6**)	44.6 ± 2.11	11.3 ± 0.37
oxypaeoniflorin (**7**)	>50	>500
nudanpinoside H (**8**)	>50	>500
α-benzoyloxypaeoniflorin (**9**)	>50	>500
β-benzoyloxypaeoniflorin (**10**)	>50	>500
paeoniflorigenone (**11**)	>50	>500
6-methoxypaeoniflorigenone (**12**)	>50	>500
paeonol (**13**)	>50	>500
paeonolide (**14**)	>50	>500
paeonoside (**15**)	>50	>500
apiopaeonoside (**16**)	>50	>500
palbinone (**17**)	11.4 ± 1.28	>500
30-norhederagenin (**18**)	28.8 ± 1.15	>500
3,3-tetramethyleneglutaric acid[b]	31.8 ± 1.78	
aminoguanidine[b]		1026.8 ± 14.58

[a] IC_{50} values were calculated from dose-dependent inhibition curves. Inhibitory effects are displayed as mean ± SE of triplicate experiments. [b] Positive control compound.

Compound **3** was obtained from the butanol fraction, and HPLC qualitative analysis was used to determine whether it was an artifact. The retention time (t_R, min) for **3** (98.9 min) was consistent with a peak (t_R of 98.3 min) in the HPLC chromatogram of the butanol fraction, but not to any peak in the methanol extract. These results suggest that **3** is the result of an extraction artifact rather than a true natural product (data not shown).

Previous research has reported the same configuration for all the known compounds in this class. It is thus likely that compounds **1** and **3** possess the same absolute configuration.[23,25−28]

An assay was carried out for the inhibition of RLAR and AGE using the MeOH extract and subsequent partitions with *n*-hexane, EtOAc, and *n*-BuOH. 3,3-Tetramethyleneglutaric (TMG), a dicarboxylic acid, can retard the cataractous process by inhibiting aldose reductase.[29] In addition, aminoguanidine (pimagedine, AG) can inhibit AGE formation, which prevents diabetic complications, experimental diabetic nephropathy, retinopathy, and neuropathy.[30] TMG and AG were used as positive controls. Inhibition of RLAR was observed using the EtOAc fraction (IC_{50} of 3.2 μg/mL) and the *n*-BuOH fraction (IC_{50} of 6.3 μg/mL), which compared favorably against TMG (IC_{50} of 5.3 μg/mL) (Table 2). The EtOAc and *n*-BuOH fractions inhibited AGE formation with respective IC_{50} values of 1.8 and 3.5 μg/mL, which are appropriately 41 and 21 times stronger than AG (76.0 μg/mL) (Table 2). Therefore, we tested the 17 compounds (except for paeonol, **13**) obtained from the *n*-hexane fraction (Table 3). The most potent inhibitor of RLAR was **17**, with an IC_{50} of 11.4 μM. Compound **18** (IC_{50} of 28.8 μM) was also more potent than TMG (31.8 μM). Features of **17** that may explain its inhibitory properties include the lack of ring E and the side chain of ring D as well as the existence of two pairs of double bonds and a conjugated carbonyl group on the D ring. It was found that **17** and **18** did not inhibit AGE (Table 3). Of the monoterpene glycosides (**1**, **3**−**10**), **3** and **6** inhibited RLAR (IC_{50} of 36.2 and 44.6 μM, respectively) and AGE (IC_{50} of 10.8 and 11.5 μM, respectively), with almost 100 times more potency than AG (IC_{50} of 1026.8 μM). AGE inhibition was enhanced by the three adjacent hydroxy groups in the benzoyl moiety connected to the sugar unit of benzoylpaeoniflorin and the butyl unit attached to the C-4 of paeoniflorin. The other monoterpene glycosides (**1**, **4**, **5**, and **7**−**10**), monoterpenes (**11** and **12**), and acetophenone glycosides (**13**−**16**) did not inhibit RLAR or AGE (Table 3). This is the first report of RLAR and AGE inhibition by compounds isolated from Moutan cortex.

The AR-related polyol pathway and AGE formation contribute to diabetic complications.[31,41] While ARIs and AGE-Is can treat diabetic complications, their side effects can be problematic.[32,33] Plant therapies may offer the benefits of multiple active components with reduced toxicity.[34] It is of interest to note that no new antidiabetic drugs derived from higher plants have been introduced to the market, with the exception of the traditional drug metformin, which is based on the structure of galegine (a component of the European antidiabetic herb *Galeda officinalis* L.).[34] Therefore, there is significant potential for new antidiabetic drugs to be found from natural sources. An Et_2O extract of Cortex moutan was found to inhibit AR,[35] and isolated compounds such as paeoniflorin,[36] polysaccharide-2b,[37] and paeonol[10] have also exhibited antidiabetic effects in several *in vitro* and *in vivo* antidiabetic models.[10,35,37] Here, the EtOAc and *n*-BuOH fractions, as well as many active compounds (**1**, **3**, **6**, **17**, and **18**), inhibited RLAR and AGE formation. These compounds may be useful as antidiabetic agents. In conclusion, this study suggests that Moutan cortex, particularly certain active components, may be a useful treatment for diabetic complications (**1**, **3**, **6**, **17**, and **18**).

Experimental Section

General Experimental Procedures. Melting points were determined on an Electrothemal apparatus. Optical rotations were measured on a JASCO DIP-370 (Tokyo, Japan) automatic digital polarimeter. UV spectra were acquired in MeOH using a JASCO V-550 UV/vis spectrometer. IR spectra were recorded on a JASCO 100 IR spectrophotometer. The NMR [^{1}H (300 MHz), ^{13}C (75 MHz), and DEPT-90 and 135 (75 MHz)] spectra were recorded on a Bruker-DRX-300 instrument. 2D NMR (HMQC, HMBC, ROESY) experiments were performed on a Bruker Advance 500 spectrometer. Chemical shifts were reported in ppm downfield from TMS, with *J* in Hz. Mass spectra were obtained with a JEOL JMS-700 Mstation mass spectrometer. Analytical TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness) and RP-18 F_{254} (Merck) plates (0.25 mm layer thickness). UV spots were visualized using ultraviolet irradiation (at 254 and 365 nm) and by spraying with 10% H_2SO_4, followed by heating with a heat gun. Column chromatography was performed on silica gel (70−230 and 230−400 mesh, Merck), YMC RP-18 resin (30−50 μm, Fuji Silysia Chemical Ltd.), and Sephadex LH-20 columns (Amersham Biosciences, Uppsala, Sweden). Preparative and analytic HPLC separations were performed on a Shimadzu SCL-10A system, which was controlled by an LC-10AD pump. This device was equipped with an SPD-10A UV−vis multiwavelength detector, a

reversed-phase Waters Spherisorb S5 ODS2 column (10 × 250 mm, Milford, MA), and a reversed-phase HICHROM C18 column (HI-5C18-250A, 4.6 × 250 mm, 5 μm, Berkshire, U.K.). Fluorescence spectra were measured with a spectrofluorometric detector (BioTek, Synergy HT, Winooski, VT).

Plant Material. The Moutan cortex (*P. suffruticosa*) was purchased from a traditional herbal market in Daejeon, Korea, in January 2007. A specimen of the plant (CNU-1554) has been verified by one of the authors (K.H.B.) and deposited in the Herbarium of the College of Pharmacy, Chungnam National University.

Extraction and Isolation. Moutan cortex (20 kg) was extracted three times with MeOH at 60 °C. The combined filtrates were evaporated to dryness *in vacuo* at 40 °C. The residue (3000 g) was stirred with 1000 mL of 95% MeOH, suspended in H_2O, and partitioned with *n*-hexane, EtOAc, *n*-BuOH, and H_2O, successively.

The *n*-hexane layer (152 g) was separated over silica gel using *n*-hexane−EtOAc (80:1) to obtain pure crystalline paeonol (**13**, 72 000 mg). The EtOAc layer (600 g) was separated with silica gel CC using increasing polarity solvents ($CHCl_3$−MeOH, 100:0, 97:3, and 0:100) to give six fractions (1−6). Repeated CC of fraction 4 (11.2 g) with silica gel (*n*-hexane−EtOAc, 6:1) gave five subfractions (4.1−4.5). Fraction 4.4 (1.8 g) was separated by silica gel CC ($CHCl_3$−MeOH, 30:1) to generate palbinone (**17**, 300 mg) and 30-norderagenin (**18**, 17 mg). Fraction 5 (9.0 g) was eluted through a Sephadex LH-20 column (MeOH−H_2O, 2:1) to obtain six fractions (5.1−5.6). Rechromatography of fraction 5.3 (7.1 g) on a silica gel column with $CHCl_3$−MeOH (20:1) generated six fractions (5.3.1−5.3.6). Fraction 5.3.1 (3.5 g) was further separated by a YMC column with MeOH−H_2O (1:1) to give 10 subfractions (5.3.1.1−5.3.1.10). Fraction 5.3.1.2 (2.1 g) was separated by C-18 CC with MeOH−H_2O (1:1) to afford **1** (72 mg) and 6-methoxypaeoniflorigenone (**12**, 1100 mg). Repeated RP preparative HPLC on Waters Spherisorb S5 ODS2 10 × 250 mm (ACN−H_2O with 0.1% formic acid, 35:75; flow rate of 0.9 mL/min) of fraction 5.3.1.7 (0.2 g) gave **2** (8 mg), α-benzoyloxypaeoniflorin (**9**, 20 mg), and β-benzoyloxypaeoniflorin (**10**, 25 mg).

The residue of the EtOAc fraction was eluted with MeOH (fr. 6), combined with the *n*-BuOH fraction (250 g), and successively subjected to CC on silica gel with $CHCl_3$−MeOH eluent ratios of 19:1, 9:1, 17:3, and pure MeOH, to give fractions B.1−B.4. Rechromatography of the second fraction (63 g) on silica gel columns using $CHCl_3$−H_2O (35:1) gave five subfractions (B.2.1−2.5). A large fraction (B.2.1, 28 g) was applied to a dry pack silica gel column prior to elution with *n*-hexane−EtOAc (4:1). Fractions containing mainly benzoylpaeoniflorin were combined and reduced to dryness *in vacuo* at 40 °C. The residue was then separated using YMC CC in MeOH−H_2O (1:1) to generate benzoylpaeoniflorin (**5**, 6.8 g). Purification of B.2.2 (2.7 g) using YMC CC with MeOH−H_2O (1:2) as an eluent yielded paeoniflorigenone (**11**, 190 mg). Mudanpinoside H (**8**, 5.7 mg) was separated from part of B.2.4 (0.12 g) by an RP preparation. HPLC was performed with a Water Spherisorb S5 ODS2 using MeCN−H_2O (1:4). B.3 (127 g) was repeatedly subjected to silica gel CC using $CHCl_3$−MeOH (20:1) as an eluent to give five subfractions (B.3.1−3.5). Fraction B.3.1 (49 g) was subjected to silica gel CC and eluted using $CHCl_3$−MeOH mixtures to afford six fractions (B.3.1.1−3.1.6). Galloylpaeoniflorin (**6**, 200 mg) was obtained from fraction B.3.1.3 (2.6 g) using RP-YMC CC (MeOH−H_2O, 1:3), and fractions B.3.1.1−3.1.6 were collected. Compound **3** (14 mg) was separated from fraction B.3.1.3.4 (40 mg) by an HPLC separation using a Water Spherisorb S5 ODS2 10 × 250 mm with ACN−H_2O (3:7), and fractions B.3.1.3.1, B.3.1.3.2, and B.3.1.3.4 were collected. A large fraction (B.3.1.5, 21.3 g) was further separated by Sephadex LH-20 (MeOH−H_2O, 2:1), silica gel ($CHCl_3$−MeOH−H_2O, 5:1:0.1), and YMC CC (MeOH−H_2O, 1:3), which generated paeoniflorin (**4**, 6.8 g), paeonolide (**14**, 50 mg), paeonoside (**15**, 1.2 g), and apiopaeonoside (**16**, 100 mg). The third fraction (B.3.3, 38 g) was repeatedly applied to a Sephadex LH-20 column prior to elution with MeOH−H_2O (2:1) to give four fractions (B.3.3.1−3.3.4). The first fraction (B.3.3.1, 17 g) was further separated by silica gel CC ($CHCl_3$−MeOH, 5:1) and YMC CC (MeOH−H_2O, 2:1) and yielded oxypaeoniflorin (**7**, 1.2 g). The major constituents of the extract were analyzed and identified. Fraction numbers that were not specifically mentioned contained only minor amounts of the numbered compounds.

HPLC Quantitative Analysis. Moutan cortex (100 mg) was extracted with 98% MeOH at room temperature (3 × 24 h). After filtration, concentration of the combined MeOH solutions under reduced pressure (at 40 °C) yielded 22 mg of extract. This extract was immediately analyzed by HPLC (a reversed-phase HICHROM column, C18 (HI-5C18-250A, 4.6 × 250 mm, 5 μm), using 0.1% formic acid in pure H_2O and MeCN as the solvent with a flow rate of 0.5 mL/min and detection at 230 nm. The BuOH fraction and compound **3** were also analyzed in the same manner. The retention time of **3** was compared to the retention times of all of the peaks that appeared in the HPLC chromatograms of the MeOH and BuOH fractions.

8-*O*-Benzoylpaeonidanin (1): white, amorphous powder; mp 107−108 °C; $[\alpha]^{25}_D$ −13.1 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.02), 241 (4.39), 250 (4.17), 284 (3.28), 291 (3.17); IR (KBr) ν_{max} 3419 (OH), 2903, 1716 (C=O), 1607, 1452 (C=C), 1316, 1280, 1158 (C−O), 1071, 1026 (glycosidic C−O), 713 cm^{-1}; HRFABMS *m/z* 579.5599 $[M - H_3O]^-$ (calcd *m/z* for $C_{31}H_{34}O_{12}$ 579.5499); ^1H NMR and ^{13}C NMR data, see Table 1.

5-Hydroxy-3*S*-hydroxymethyl-6-methyl-2,3-dihydrobenzofuran (2): white powder; mp 90−91 °C; $[\alpha]^{25}_D$ +6.7 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.94), 250 (3.17), 300 (3.28), 315 (3.51) nm; IR (KBr) ν_{max} 3348, 3164 (OH), 2954, 1478 (C=C), 1197, 1161, 1051, (C−O), 877 cm^{-1}; ^1H NMR (300 MHz, methanol-d_4) δ 2.11 (3H, s, H-11), 3.48 (1H, m, H-3), 3.54 (1H, dd, *J* = 10.2, 7.8 Hz, H-10a), 3.69 (1H, dd, *J* = 10.2, 5.4 Hz, H-10b), 4.33 (1H, dd, *J* = 8.7, 5.2 Hz, H-2a), 4.51 (1H, dd, *J* = 9.0, 8.7 Hz, H-2b), 6.46 (1H, s, H-7), 6.66 (1H, s, H-4); ^{13}C NMR (75 MHz, methanol-d_4) δ 16.8 (C-11), 46.7 (C-3), 65.8 (C-10), 75.4 (C-2), 111.9 (C-7), 112.6 (C-4), 125.7 (C-6), 126.8 (C-9), 150.2 (C-5), 154.9 (C-8); HRESIMS *m/z* 180.0786 $[M]^+$ (calcd *m/z* for $C_{10}H_{12}O_3$ 180.0786).

4-*O*-Butylpaeoniflorin (3): white foam; mp 173−175 °C; $[\alpha]^{25}_D$ −7.8 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 200 (4.41), 210 (4.52), 233 (4.31), 240 (3.14), 250 (3.96) nm; IR (KBr) ν_{max} 3396 (OH), 2932, 1712 (C=O), 1600, 1451 (C=C), 1281, 1229 (C−O), 1075, 955 (glycosidic C−O), 715 cm^{-1}; HRESIMS *m/z* 536.2234 $[M]^+$ (calcd *m/z* for $C_{27}H_{36}O_{11}$ 536.2258); ^1H NMR and ^{13}C NMR data, see Table 1.

Acid Hydrolysis of 1 and 3. Compounds **1** and **3** (each 3 mg) were dissolved in 1.0 N HCl (dioxane−H_2O, 1:1, v/v, 1.0 mL) and then heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with $AgCO_3$, and the solvent was removed using N_2 gas overnight. After extraction with $CHCl_3$, the aqueous layer was concentrated to dryness using N_2 gas. The residue was dissolved in 0.1 mL of dry pyridine, and L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL) was added to the resulting solution. The reaction mixture was heated to 60 °C for 2 h. After 0.1 mL of trimethylsilylimidazole solution was added, the reaction was heated to 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (each 0.1 mL), and the organic layer was analyzed by gas liquid chromatography using an SPB-1 column (0.25 mm × 30 m) and an FID detector, with a column temperature of 210 °C, injector temperature of 270 °C, detector temperature of 300 °C, and He as the carrier gas (30 mL/s).[38] A peak corresponding to D-glucose from the hydrolysate of **1** was detected at a t_R of 14.12 min. A peak corresponding to D-glucose from the hydrolysate of **3** was detected at 14.11 min. The retention times for the authentic samples (obtained from Sigma), after similar treatments, were 14.11 min (D-glucose) and 14.26 min (L-glucose). Co-injection of the hydrolysates of **1** (or **3**) with D-glucose gave a single peak.

Assay for Aldose Reductase and Inhibition of Advanced Glycation End-Product Formation. Measement of RLAR Activity. Rat lenses were removed from the eyes of 8-week-old Sprague−Dawley rats (Orient Bio Link, Co., Sungnam, Korea) weighing 250−280 g. The lenses were homogenized in 12 volumes of 135 mM Na/K phosphate buffer (pH 7.0) containing 0.5 mM phenylmethanesulfonyl fluoride and 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 14 000 rpm for 30 min, and the supernatant was used as a crude rat-lens aldose reductase (RLAR). The RLAR activity was assayed according to previously reported methods with slight modification.[35,36] The incubation mixture (1.0 mL in total) contained 135 mM Na/K phosphate buffer (pH 7.0), 100 mM Li_2SO_4, 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate, and an enzyme fraction (50 μL), with or without the sample solution (25 μL). The reaction was initiated by the addition of NADPH at 37 °C and stopped by the addition of 0.5 M aqueous HCl (0.3 mL). To convert NADP to a fluorescent product, a 6 M aqueous NaOH solution containing 10 mM 1*H*-imidazole (1 mL) was added, and the mixture was heated to

60 °C for 10 min. The fluorescence was measured with a spectrofluorometric detector (BioTek, Synergy HT) at excitation and emission wavelengths of 360 and 460 nm, respectively. The RLAR assays were performed in triplicate to yield measures of the SE. The concentration of each test sample giving rise to 50% inhibition of activity (IC_{50}) was estimated from the least-squares regression line of the logarithmic concentration plotted against the remaining activity.

Determination of Advanced Glycation End-Product Formation. According to an established method,[33] the reaction mixture, containing 700 μL of bovine serum albumin (BSA, 10 mg/mL; Sigma) in 50 mM phosphate buffer (pH 7.4) with 0.02% sodium azide, was added to 0.2 M fructose and glucose (100 μL). In screw-cap tubes (1.5 mL), the reaction mixture was mixed with 200 μL of serially diluted compounds or aminoguanidine (obtained from Sigma) as positive control. The tubes were incubated at 37 °C for 14 days. The fluorescent reaction products (200 μL) were transferred to 96-well plates and assayed on a spectrofluorometric detector (BioTek; Synergy HT) at excitation and emission wavelengths of 350 and 450 nm, respectively.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) The Diabetes Control and Complication Trial Research Group. *N. Engl. J. Med.* **1993**, *329*, 997−986.
(2) Williamson, J.-R.; Chang, K.; Frangos, M.; Hasan, K.-S.; Ido, Y.; Kawamura, T.; Nyengaard, J.-R.; Van den Enden, M.; Kilo, C.; Tilton, R.-G. *Diabetes* **1993**, *42*, 801–813.
(3) Brownlee, M. *Natural* **2001**, *414*, 813–820.
(4) Beyer-Mears, A.; Cruz, E. *Diabetes* **1985**, *34*, 15–21.
(5) Kalousova, M.; Zima, T.; Tesar, V.; Stipek, S.; Sulkova, S. *Kidney Blood Press. Res.* **2004**, *27*, 18–28.
(6) Forbes, J.-M.; Cooper, M.-E.; Oldfield, M.-D.; Thomas, M.-C. *J. Am. Soc. Nephrol.* **2003**, S254–258.
(7) Ding, H.-Y.; Wu, Y.-C.; Lin, H.-C.; Chan, Y.-Y.; Wu, P.-L.; Wu, T.-S. *Chem. Pharm. Bull.* **1999**, *47*, 652–655.
(8) Shimizu, M.; Zenko, Y.; Tanaka, R.; Matsuzawa, T.; Morita, N. *Chem. Pharm. Bull.* **1993**, *48*, 1469–1471.
(9) Hong, H.; Wang, Q.; Zhao, Z.; Liu, G.; Shen, Y.; Chen, G. *Yao Xue Xue Bao* **2003**, *38*, 255–259.
(10) Lau, C.-H.; Chan, C.-M.; Chan, Y.-W.; Lau, K.-M.; Lau, T.-W.; Lam, F.-C.; Law, W.-T.; Che, C.-T.; Leung, P.-C.; Fung, K.-P.; Ho, Y.-Y.; Lau, C.-B.-S. *Phytomedicine* **2007**, *14*, 778–784.
(11) Yoshikawa, M.; Ohta, T.; Kawaguchi, A.; Matsuda, H. *Chem. Pharm. Bull.* **2000**, *48*, 1327–1331.
(12) Lemmich, J. *Phytochemistry* **1996**, *41*, 1337–1340.
(13) Kaneda, M.; Iitaka, Y.; Shibata, S. *Tetrahedron* **1972**, *28*, 4309–4011.
(14) Shimizu, M.; Hayashi, T.; Morita, N.; Kiuchi, F.; Noguchi, H.; Sankawa, U. *Chem. Pharm. Bull.* **1983**, *31*, 577–583.
(15) Lin, H.-C.; Ding, H.-Y.; Wu, T.-S.; Wu, P.-L. *Phytochemistry* **1996**, *41*, 237–242.
(16) Ryu, G.; Park, E.-K.; Joo, J.-H.; Lee, B.-H.; Choi, B.-W.; Jung, D.-S.; Lee, N.-H. *Arch. Pharm. Res.* **2001**, *24*, 105–108.
(17) Fukuhara, Y.; Yoshida, D. *Biol. Chem.* **1987**, *51*, 1441–1442.
(18) Matsuda, H.; Ohta, T.; Kawaguchi, A.; Yoshikawa, M. *Chem. Pharm. Bull.* **2001**, *49*, 69–72.
(19) Kuwajima, H.; Shibano, N.; Baba, T.; Takaishi, K.; Inoue, K.; Shingu, T. *Phytochemistry* **1996**, *41*, 289–292.
(20) Kadota, S.; Terashima, S.; Basnet, P.; Kikuchi, T.; Namba, T. *Chem. Pharm. Bull.* **1993**, *41*, 487–489.
(21) Ikuta, A.; Itokawa, H. *Phytochemistry* **1988**, *27*, 2813–2815.
(22) Aimi, N.; Inaba, M.; Watanabe, M.; Shibata, S. *Tetrahedron* **1969**, *25*, 1825–1838.
(23) Kostova, I.-N.; Simeonov, M.-F.; Todorova, D.-I.; Petkova, P.-L. *Phytochemistry* **1998**, *48*, 511–514.
(24) Dong, Z.; Karpowicz, R. J.; Bai, J. S.; Yap, G. A.; Fox, J. M. *J. Am. Chem. Soc.* **2006**, *128*, S-7−8.
(25) Okasaka, M.; Kashiwada, Y.; Kodzhimatov, O. K.; Ashurmetov, O.; Takaishi, Y. *Phytochemistry* **2008**, *69*, 1767–1772.
(26) Yoshikawa, M.; Harada, E.; Kawaguchi, A.; Yamahara, J. *Chem. Pharm. Bull.* **1993**, *41*, 630–632.
(27) Wu, S.-H.; Luo, X.-D.; Ma, Y.-B.; Hao, X.-J.; Wu, D.-G. *J. Asian Nat. Prod. Res.* **2002**, *4*, 135–140.
(28) Kostova, I.-N.; Simeonov, M.-F.; Todorova, D.-I.; Petkova, P.-L. *Phytochemistry* **1998**, *48*, 511–514.
(29) Jedziniak, J. A.; Kinoshita, J. H. *Invest Ophthal. Vis. Sci.* **1971**, *10*, 357–366. (a) Kinoshita, J. H. *Invest. Ophthalmol. Vis. Sci.* **1974**, *13*, 713–724.
(30) Thornalley, P. J. *Archiv. Biochem. Biophys.* **2003**, *419*, 31–40.
(31) Dunlop, M. *Kidney Intern.* **2000**, *58*, S-3–S-12.
(32) de la Fuente, J. A.; Manzanaro, S.; Martín, M. J.; de Quesada, T. G.; Reymundo, I.; Luengo, S. M.; Gago, F. *J. Med. Chem.* **2003**, *46*, 5208–5221.
(33) Preifer, M. A.; Schumer, M. P.; Geiber, D. A. *Diabetes* **1997**, *46*, S82.
(34) Yoshikawa, M.; Matsuda, H. *Traditional Medcines for Modern Time Antidiabetic Plants*; Soumyanath, A., Ed.; CRC: Taylor & Francis Group, 2007.
(35) Suzuki, K.; Goto, A.; Ishige, Y.; Komatu, J.; Kamei, J. *Jpn. J. Pharmacol.* **1998**, *78*, 87–91.
(36) Hsu, F.-L.; Lai, C.-W.; Cheng, J.-T. *Planta Med.* **1997**, *63*, 323–325.
(37) Hong, H.; Wang, Q.-M.; Zhao, Z.-P.; Liu, G.-Q.; Shen, Y.-S.; Chen, G.-L. *Acta Pharm. Sin.* **2003**, *38*, 255–259.
(38) Ahn, M. J.; Kim, C. Y.; Ryu, M. R.; Cheong, J. H.; Chin, Y. W.; Kim, J. W. *J. Nat. Prod.* **2006**, 360–364.
(39) Dufrane, S.-P.; Malaisse, W.-J.; Sener, A. *Biochem. Med.* **1984**, *32*, 99–105.
(40) Kim, H.-Y.; Oh, J.-H. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 184–88.
(41) Vinson, J.-A.; Howard, T.-B., III. *J. Nutr. Biochem.* **1996**, *7*, 659−663.

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