Bioactive Constituents from Chinese Natural Medicines. XV.[1] Inhibitory Effect on Aldose Reductase and Structures of Saussureosides A and B from *Saussurea medusa*

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The 80% aqueous acetone extract from the whole plant of *Saussurea medusa* MAXIM. was found to inhibit rat lens aldose reductase (IC_{50}=1.4 μg/ml). From this extract, flavonoids, lignans, and quinic acid derivatives were isolated together with two new ionone glycosides, saussureosides A and B. Their absolute stereostructures were elucidated on the basis of chemical and physicochemical evidence including the application of modified Mosher's method. In addition, some isolates were found to show an inhibitory effect on aldose reductase.

Key words *Saussurea medusa*; aldose reductase inhibitor; saussureoside; flavonoid; ionone glycoside

The Compositae plant, *Saussurea medusa* MAXIM., is a rare perennial medicinal herb growing in the northwestern parts of China (*e.g.*, Tibet, Xinjiang, Qinghai, Gansu, Yunnan, and Sichuan provinces). The whole plant or aerial parts of *S. medusa*, popularly known as "Xuelianhua (雪蓮花)", has been commonly used in both traditional Chinese medicine and Tibetan folk medicine as a remedy for rheumatic arthritis, menoxenia, gynopathy, traumatic bleeding, and headache.[2—4] Several lignan, neolignan, flavonoid, terpenoid, and chlorophyll constituents have previously been isolated from *S. medusa*[5—10] and their pharmacologic activities such as anti-tumor-promoting,[5] immunosuppressive[6] and cell-protecting activities[7] were also reported. In the course of our studies on bioactive constituents from Chinese natural medicines,[11—17] we found that the 80% aqueous acetone extract from the whole plant of *S. medusa* showed a potent inhibitory effect on rat lens aldose reductase (IC_{50}=1.4 μg/ml). In this paper, we report the isolation and structure elucidation of two new megastigmane glycosides, saussureosides A (**1**) and B (**2**), together with 13 flavonoids (**3**—**15**), four lignans (**16**—**19**) and three neolignans (**20**—**22**), three quinic acids (**23**—**25**), and 24 other known constituents.

The 80% aqueous acetone extract from the whole plant of *S. medusa* was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish the EtOAc–soluble fraction and aqueous layer. The aqueous layer was extracted with *n*-butanol (*n*-BuOH) to give *n*-BuOH and H_2O-soluble fractions. The *n*-BuOH-soluble fraction, which was the most active fraction (IC_{50}=0.8 μg/ml), was subjected to normal-phase and reversed-phase silica gel column chromatography and repeated HPLC to give saussureosides A (**1**, 0.0007%) and B (**2**, 0.0038%), apigenin 7-*O*-β-D-glucopyranoside[18] (**4**, 0.0016%), apigenin 4′-*O*-β-D-glucopyranoside[19] (**5**, 0.0089%), apigenin 7-*O*-rutinoside[20] (**6**, 0.0054%), luteolin 7-*O*-β-D-glucopyranoside[20] (**8**, 0.034%), luteolin 4′-*O*-β-D-glucopyranoside[19] (**9**, 0.042%), luteolin 7-*O*-rutinoside[20] (**10**, 0.023%), chrysoeriol 7-*O*-β-D-glucopyranoside[21] (**11**, 0.0034%), chrysoeriol 7-*O*-rutinoside[22] (**12**, 0.0030%), quercetin 3-*O*-β-D-glucopyranoside[23] (**14**, 0.079%), isorhamnetin 3-*O*-rutinoside[24] (**15**, 0.0035%), (+)-pinoresinol *O*-β-D-glucopyranoside[25] (**17**, 0.0026%), (+)-syringaresinol *O*-β-D-glucopyranoside[26] (**19**, 0.0011%), arctiin[27] (**22**, 0.062%), 3-*O*-caffeoylquinic acid methyl ester[28,29] (**23**, 0.0011%), 4-*O*-caffeoylquinic acid methyl ester[28] (**24**, 0.0012%), 5-*O*-caffeoylquinic acid methyl ester[30] (**25**, 0.019%), syringin[31] (0.0063%), junipediol A[32] (0.0011%), phenethyl *O*-β-D-glucopyranoside[33] (0.0007%), benzyl *O*-β-D-glucopyranoside[34] (0.0028%), *p*-anisyl *O*-β-D-glucopyranoside[35] (0.0006%), (*Z*)-3-hexenyl *O*-β-D-glucopyranoside[36] (0.0010%), L-phenylalanine[37] (0.0018%), thymidine[37] (0.0026%), and sitosterol 3β-*O*-β-D-glucopyranoside[29,38] (0.0046%). The EtOAc-soluble fraction was also subjected to normal-phase and reversed-phase silica gel column chromatography and repeated HPLC to give apigenin[23] (**3**, 0.0021%), luteolin[23] (**7**, 0.0069%), quercetin[23] (**13**, 0.0060%), (+)-pinoresinol[39] (**16**, 0.0004%), (+)-syringaresinol[40] (**18**, 0.0004%), (−)-secoisolariciresinol[41] (**20**, 0.0007%), (−)-arctigenin[27] (**21**, 0.038%), **22** (0.054%), 3-methoxy-4-hydroxyphenylethanol[42] (0.0021%), (−)-2-oxoisodauc-5-en-12-al[43] (0.0002%), (+)-glutinol[44] (0.0006%), α-amyrin[45] (0.0086%), lupeol[45] (0.0074%), clionasterol[46] (0.0026%), azelaic acid[37] (0.0024%), linolenic acid[37] (0.027%), linoleic acid[37] (0.065%), oleic acid[37] (0.0093%), hexadecanoic acid[37] (0.075%), octadecanoic acid[37] (0.0092%), eicosanoic acid[37] (0.0028%), docosanoic acid[37] (0.0025%), tricosanoic acid[37] (0.0007%), and eicosanol[37] (0.0011%).

Structure of Saussureosides A (1) and B (2) Saussureoside A (**1**) was isolated as a white powder with negative optical rotation ($[\alpha]_D^{24}$ −45.0°). In the UV spectrum of **1**, an absorption maximum was observed at 229 (log ε 4.07) nm, while the IR spectrum of **1** showed absorption bands at 1684, 1671, and 1260 cm^{-1} assignable to α,β-unsaturated carbonyl, olefin, and epoxide functions and strong absorption bands at 3410 and 1078 cm^{-1} suggestive of a glycoside moiety. In the positive-ion FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 425 $(M+Na)^+$. The molecular formula $C_{19}H_{30}O_9$ of **1** was determined from the quasimolecular ion peak observed in the FAB-MS and by high-resolution FAB-MS measurement. The ^{1}H-NMR (pyridine-d_5, Table 2) and

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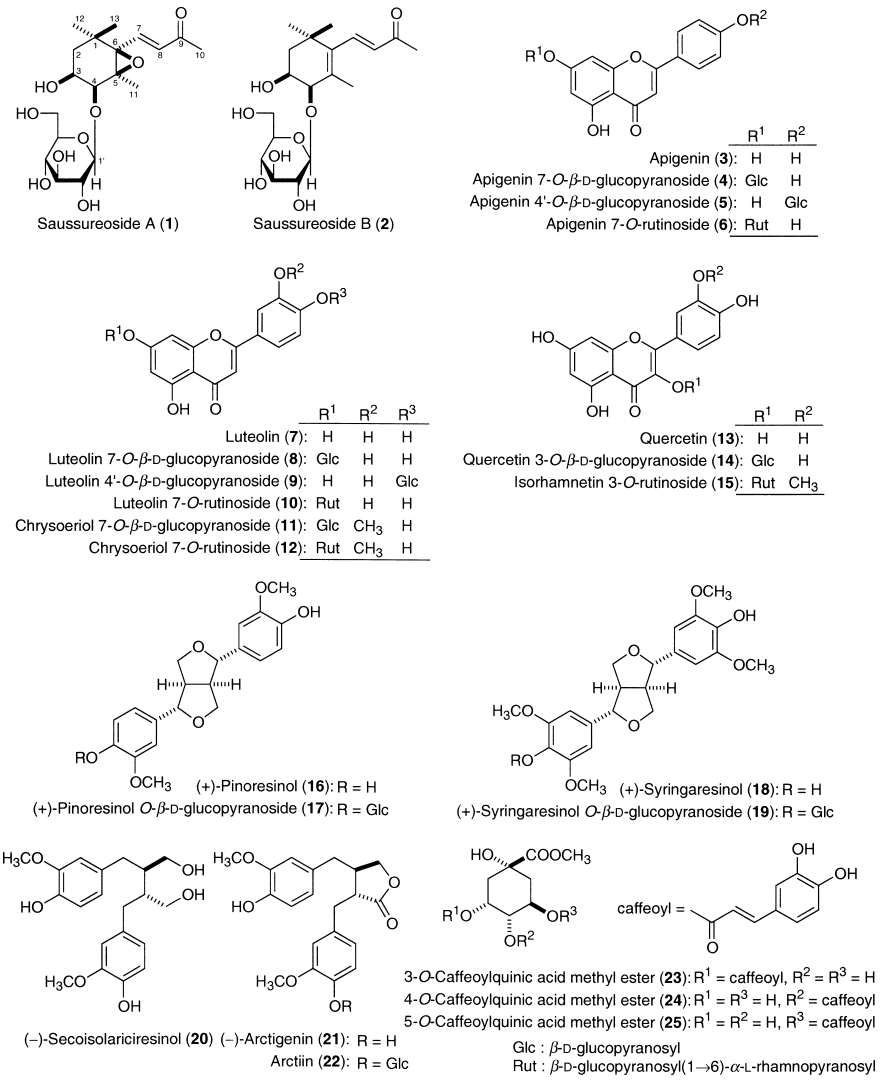

Chart 1

Table 1. Inhibitory Effects of 80% Aqueous Acetone Extract and EtOAc-, *n*-BuOH-, H_2O-Soluble Fractions from *Saussurea medusa* on Rat Lens Aldose Reductase

	IC_{50} (μg/ml)
80% Aqueous acetone extract	1.4
EtOAc-soluble fraction	1.4
n-BuOH-soluble fraction	0.8
H_2O-soluble fraction	2.2

^{13}C-NMR (Table 3) spectra[47] of **1** showed signals assignable to four methyls [δ 0.98, 1.02, 1.67, 2.24 (all s, 12, 13, 11, 10-H_3)], a methylene [δ 1.49 (dd, J=3.4, 12.5 Hz), 1.94 (dd, J=12.2, 12.5 Hz), 2-H_2], two methines bearing an oxygen function [δ 4.25 (ddd, J=3.4, 3.4, 12.2 Hz, 3-H), 4.40 (d, J=3.4 Hz, 4-H)], and a *trans*-olefin pair [δ 6.47, 7.03 (both d, J=15.9 Hz, 8, 7-H)] together with a β-glucopyranosyl part [δ 5.00 (d, J=6.4 Hz, 1′-H)]. Acid hydrolysis of **1** with 1 M hydrochloric acid (HCl) liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[1,13—15,17,48] The aglycon **1a** was obtained by enzymatic hydrolysis of **1** with β-glucosidase. As shown in Fig. 1, the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) experiment on **1** indicated the presence of partial structures written in bold lines, and in the heteronuclear multiple-bond correlations (HMBC) experiment, long-range correlations were observed between the following protons and carbons (2-H_2, 12-H_3, 13-H_3 and 1-C; 4-H, 11-H_3 and 5-C; 4-H, 7-H, 12-H_3, 13-H_3 and 6-C; 7-H, 8-H, 10-H_3 and 9-C; 1′-H and 4-C). Furthermore, comparison of the ^{13}C-NMR data of **1** with those of **1a** revealed a glycosylation shift around the 4-position in **1**. Thus, the connectivity of the β-D-glucopyranosyl moiety in **1** was clarified to be the 4-position of **1a**. The relative stereostructure of the aglycon part (**1a**) in **1** was characterized by nuclear Overhauser enhancement spectroscopy (NOESY) experiment, which showed NOE correlations between the following proton pairs (2α-H and 3-H; 2β-H and 13-H_3; 3-H and 4-H; 11-H_3 and 4-H, 7-H_2) as shown in Fig. 1. Finally, the absolute configurations of **1** and **1a** were characterized by application of the modified Mosher's method.[49] Namely, treatment of **1a** with (*R*)- or (*S*)-2-methoxy-2-trifluoromethylphenylacetic acid [(*R*)- or (*S*)-MTPA] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC·HCl) and 4-dimethylaminopyridine (4-DMAP) selectively yielded the 3-mono-MTPA esters (**1b**, **c**), respectively,

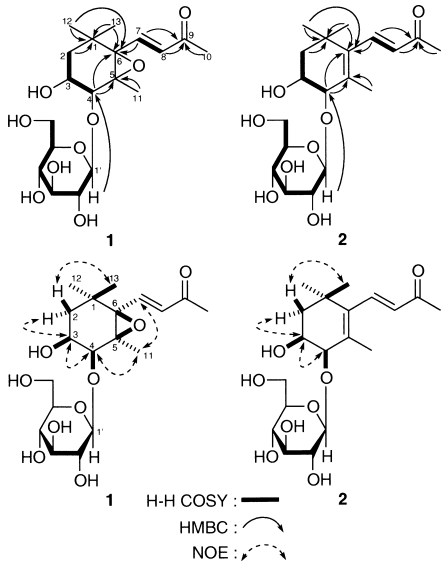

Fig. 1. H–H COSY, HMBC, and NOE Correlations of **1** and **2**

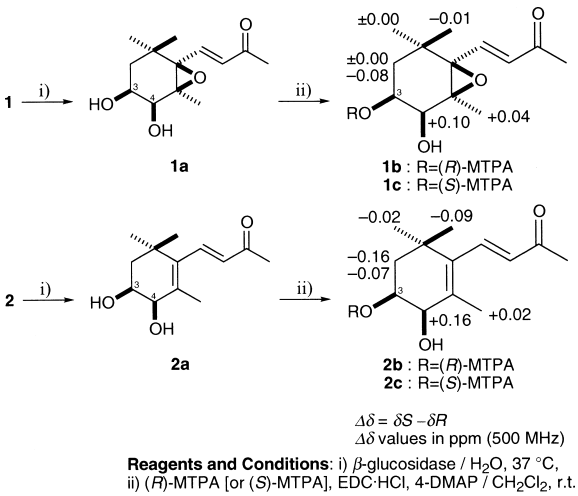

Fig. 2

whose ^{1}H-NMR data showed an acylation shift at the 3-position in **1a**. As shown in Fig. 2, the signals due to protons attached to the 4 and 11-positions in the 3-mono-(*S*)-MTPA ester (**1c**) were observed at lower fields compared with those of the 3-mono-(*R*)-MTPA ester (**1b**) [$\Delta\delta$: positive], while the signals due to protons on the 2 and 13-positions in **1c** were observed at higher fields compared with those of **1b** [$\Delta\delta$: negative]. Consequently, the absolute configuration at the 3-position of **1a** was determined to be *S* configuration and the absolute stereostructures of **1** and **1a** were elucidated as shown.

Saussureoside B (**2**) was obtained as a white powder with negative optical rotation ($[\alpha]_D^{26}$ −39.5°). The molecular formula of **2** was determined from the positive-ion FAB-MS and by high-resolution FAB-MS analysis to be $C_{19}H_{30}O_8$. In the UV spectrum of **2**, absorption maxima were observed at 227 (log ε 3.89) and 279 (log ε 3.78) nm, while the IR spectrum of **2** showed absorption bands due to hydroxyl, conjugated enone, and ether functions (3432, 1671, 1655, 1076 cm^{-1}). The ^{1}H-NMR (Table 2, pyridine-d_5) and ^{13}C-NMR (Table 3) spectra[47] of **2** showed the presence of the following functions: four methyls [δ 1.02, 1.05, 2.11, 2.27 (all s, 13, 12, 11, 10-H$_3$)], a methylene [δ 1.70 (dd, J=3.0, 12.5 Hz), 2.06 (dd, J=12.5, 12.5 Hz), 2-H$_2$], two methines bearing an oxygen function [δ 4.06 (ddd, J=3.0, 3.4, 12.5 Hz, 3-H), 4.30 (d, J=3.4 Hz, 4-H)], and a *trans*-olefin pair [δ 6.11, 7.22 (both d, J=16.5 Hz, 8, 7-H)] together with a β-glucopyranosyl part [δ 5.00 (d, J=6.4 Hz, 1′-H)]. The acid hydrolysis of **2** with 1 M HCl liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[1,13—15,17,48] The aglycon **2a** was obtained by enzymatic hydrolysis of **2** with β-glucosidase. The structures of **2** and **2a** were confirmed by ^{1}H–^{1}H COSY and HMBC experiments. That is, the ^{1}H–^{1}H COSY experiment on **2** indicated the presence of the partial structures written in bold lines, and in the HMBC experiment, long-range correlations were observed between the following protons and carbons (2-H, 12-H$_3$, 13-H$_3$ and 1-C; 4-H, 11-H$_3$ and 5-C; 4-H, 7-H, 12-H$_3$,

Table 2. ^{1}H-NMR (500 MHz, Pyridine-d_5) Data of Saussureosides A (**1**) and B (**2**)

H-	**1**	**2**
2α	1.49 dd (3.4, 12.5)	1.70 dd (3.0, 12.5)
2β	1.94 dd (12.2, 12.5)	2.06 dd (12.5, 12.5)
3	4.25 ddd (3.4, 3.4, 12.2)	4.06 ddd (3.0, 3.4, 12.5)
4	4.40 d (3.4)	4.30 d (3.4)
7	7.03 d (15.9)	7.22 d (16.5)
8	6.47 d (15.9)	6.11 d (16.5)
10	2.24 s	2.27 s
11	1.67 s	2.11 s
12	0.98 s	1.05 s
13	1.02 s	1.02 s
1′	5.00 d (6.4)	5.00 d (6.4)
2′	4.11 m	4.09 m
3′	4.26 m	4.24 m
4′	4.25 m	4.23 m
5′	4.06 m	4.05 m
6′	4.36 dd (5.2, 11.4)	4.35 dd (5.8, 11.9)
	4.58 dd (2.1, 11.4)	4.58 dd (2.5, 11.9)

δ in ppm and J (in parentheses) in Hz.

Table 3. ^{13}C-NMR (125 MHz) Data of Saussureosides A (**1**) and B (**2**), **1a**, and **2a**

C-	**1**[a)]	**1a**[b)]	**2**[a)]	**2a**[b)]
1	34.7	34.5	37.1	37.0
2	42.0	39.4	43.0	42.4
3	64.9	65.9	66.1	66.8
4	84.7	71.6	84.3	71.9
5	69.0	68.8	131.5	134.3
6	70.5	70.1	139.7	138.7
7	142.7	142.2	141.9	142.5
8	133.5	132.9	133.9	133.7
9	197.0	197.4	197.0	197.7
10	27.6	28.1	27.4	27.4
11	16.9	16.8	19.9	20.2
12	25.0	24.6	27.1	27.6
13	29.0	29.2	29.9	30.0
1′	106.6		106.9	
2′	75.1		75.0	
3′	78.5		78.3	
4′	71.5		71.4	
5′	78.7		78.7	
6′	62.4		62.4	

Measured in *a*) pyridine-d_5 and *b*) $CDCl_3$.

Table 4. Inhibitory Effects of Constituents from *Saussurea medusa* on Rat Lens Aldose Reductase

	IC_{50} (μM)
Saussuroside A (**1**)	>100 (3%)[a]
Saussuroside B (**2**)	>100 (13%)
Apigenin (**3**)	2.2[52]
Apigenin 7-*O*-β-D-glucopyranoside (**4**)	4.4
Apigenin 4′-*O*-β-D-glucopyranoside (**5**)	3.2
Apgenin 7-*O*-rutinoside (**6**)	4.7
Luteolin (**7**)	0.45[52]
Luteolin 7-*O*-β-D-glucopyranoside (**8**)	0.99[52]
Luteolin 4′-*O*-β-D-glucopyranoside (**9**)	4.8
Luteolin 7-*O*-rutinoside (**10**)	0.92
Chrysoeriol 7-*O*-β-D-glucopyranoside (**11**)	26
Chrysoeriol 7-*O*-rutinoside (**12**)	14
Quercetin (**13**)	2.2[52]
Quercetin 3-*O*-β-D-glucopyranoside (**14**)	4.5[52]
Isorhamnetin 3-*O*-rutinoside (**15**)	19[56]
(+)-Pinoresinol (**16**)	>100 (34%)
(+)-Pinoresinol *O*-β-D-glucopyranoside (**17**)	>100 (37%)
(+)-Syringaresinol (**18**)	>100 (13%)
(+)-Syringaresinol *O*-β-D-glucopyranoside (**19**)	>100 (38%)
(−)-Secoisolariciresinol (**20**)	>100 (26%)
(−)-Arctigenin (**21**)	>100 (16%)
Arctiin (**22**)	20
3-Caffeoylquinic acid methyl ester (**23**)	13
4-Caffeoylquinic acid methyl ester (**24**)	16
5-Caffeoylquinic acid methyl ester (**25**)	1.3
Epalrestat	0.072

a) Values in parentheses indicate inhibition (%) at 100 μM.

13-H_3 and 6-C; 7-H, 8-H, 10-H_3 and 9-C; 1′-H and 4-C) (Fig. 1).

The relative stereostructure of **2** was determined by a NOESY experiment, in which correlations were observed between the following proton pairs (2α-H and 3-H; 2β-H and 13-H_3; 3-H and 4-H). The absolute configurations of **2** and **2a** were determined by a modified Mosher's method.[49] As shown in Fig. 2, the 3-mono-MTPA esters (**2b** and **2c**) were derived from **2a** by a method similar to that for **1a**. The protons on the 4 and 11-positions of the (*S*)-MTPA ester (**2c**) resonated at lower fields than those of the (*R*)-MTPA ester (**2b**) [$\Delta\delta$: positive], while the protons on the 2, 12, and 13-positions of **2c** were observed at higher fields compared to those of **2b** [$\Delta\delta$: negative]. Consequently, the absolute configurations at the 3-position in **2** and **2a** were elucidated to be *S*. On the basis of this evidence, the absolute configurations of **2** and **2a** were determined as shown.[50,51]

Inhibitory Effect on Rat Lens Aldose Reductase As a key enzyme in the polyol pathway, aldose reductase has been reported to catalyze the reduction of glucose to sorbitol. Sorbitol does not readily diffuse across cell membranes, and the intracellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as cataract. Previously, we reported that various flavonoid and terpenoid constituents from several natural medicines and medicinal foods inhibited aldose reductase inhibitory activity.[52—57] Since the aqueous acetone extract from the whole plants of *S. medusa* was found to show aldose reductase inhibitory activity, the principal isolated constituents were examined (Table 4).

Among these principal constituents, flavones with the 3′,4′-dihydroxyl group [*e.g.*, luteolin 7-*O*-rutinoside (**10**, IC_{50}=0.92 μM)] showed strong activity in agreement with the previous study.[52] In contrast, lignan constituents (**16**—**22**) showed weak activity. As to the activity of caffeoylquinic acid methyl esters, 5-*O*-caffeoylquinic acid methyl ester (**25**, 1.3 μM) showed stronger activity than **23** and **24**, and this is the first report of caffeoylquinic acid methyl esters with inhibitory activity for aldose reductase.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution EI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution FAB-MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index detector.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The whole plant of *Saussurea medusa* Maxim. was purchased from Kunming, Yunnan province, China in September 2001, and the botanical identification was kindly undertaken by Dr. Seiichi Yamaji (Toyama Pharmaceutical and Medical University, Japan).[3,4] A voucher specimen (2001.09. Yunnan-01) of this plant is on file in our laboratory.

Extraction and Isolation The dried whole plant of *S. medusa* (2.70 kg) was powdered and extracted with 80% aqueous acetone three times at room temperature. Evaporation of the solvent under reduced pressure provided the aqueous acetone extract (300 g, 11.1%). This extract (286 g) was partitioned in an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction and aqueous layer. The aqueous layer was extracted with *n*-BuOH and removal of the solvents *in vacuo* from the EtOAc-, *n*-BuOH-, and H_2O-soluble fractions yielded 72 g (2.8%), 60 g (2.3%), and 154 g (6.0%) of the residues, respectively.

Normal-phase silica gel column chromatography [2.2 kg, *n*-hexane–EtOAc (50 : 1→20 : 1→10 : 1→5 : 1→2 : 1→1 : 1, v/v)→MeOH] of the EtOAc-soluble fraction (72 g) gave eight fractions [Fr. 1 (0.99 g), Fr. 2 (2.60 g), Fr. 3 (7.62 g), Fr. 4 (0.85 g), Fr. 5 (8.92 g), Fr. 6 (9.03 g), Fr. 7 (24.40 g), and Fr. 8 (16.10 g)]. Fraction 4 (0.85 g) was separated by reversed-phase silica gel column chromatography [25 g, MeOH–H_2O (70 : 30→85 : 15→98 : 2, v/v)→MeOH] to furnish four fractions [Fr. 4-1 (58 mg), Fr. 4-2 (83 mg), Fr. 4-3 (242 mg), Fr. 4-4 (459 mg)]. Fraction 4-3 (240 mg) was separated by HPLC [YMC-Pack ODS-A (YMC Co., Ltd., Kyoto, Japan, 250×20 mm i.d.), MeOH–H_2O (95 : 5, v/v)] to give (+)-glutinol (13.9 mg, 0.0006%). Fraction 5 (8.92 g) was subjected to reversed-phase silica gel column chromatography [240 g, MeOH–H_2O (75 : 25→85 : 15→98 : 2, v/v)→MeOH] to furnish four fractions [Fr. 5-1 (0.12 g), Fr. 5-2 (4.72 g), Fr. 5-3 (3.43 g), Fr. 5-4 (0.50 g)]. Fraction 5-1 (120 mg) was separated by HPLC [MeOH–H_2O (77 : 23, v/v)] to give (−)-2-oxoisodauc-5-en-12-al (5.4 mg, 0.0002%). Fraction 5-2 (630 mg) was purified by HPLC [MeOH–H_2O (90 : 10, v/v)] to give linoleic acid (118 mg, 0.035%), hexadecanoic acid (178 mg, 0.053%), and oleic acid (33.7 mg, 0.0093%). Fraction 5-3 (590 mg) was separated by HPLC [MeOH–H_2O (98 : 2, v/v)] to give linoleic acid (19.7 mg, 0.0044%), hexadecanoic acid (94.7 mg, 0.022%), octadecanoic acid (40.3 mg, 0.0092%), eicosanoic acid (12.1 mg, 0.0028%), eicosanol (4.8 mg, 0.0011%), docosanoic acid (10.8 mg, 0.0025%), tricosanoic acid (3.0 mg, 0.0007%), lupeol (31.8 mg, 0.0074%), and α-amyrin (37.2 mg, 0.0086%). Fraction 6 (9.03 g) was separated by reversed-phase silica gel column chromatography [280 g, MeOH–H_2O (75 : 25→85 : 15→98 : 2, v/v)→MeOH] to furnish four fractions [Fr. 6-1 (0.62 g), Fr. 6-2 (2.40 g), Fr. 6-3 (4.03 g), Fr. 6-4 (1.90 g)]. Fraction 6-2 (1.08 g) was separated by HPLC [MeOH–H_2O (87 : 13, v/v)] to give linolenic acid (305 mg, 0.027%) and linoleic acid (277 mg, 0.025%). Fraction 6-3 (1.05 g) was purified by HPLC [MeOH–H_2O (98 : 2, v/v)] to give clionasterol (16.6 mg, 0.0026%). Fraction 7 (24.40 g) was subjected to reversed-phase silica gel column chromatography [730 g, MeOH–H_2O (40 : 60→55 : 45→70 : 30→85 : 15→98 : 2, v/v)→MeOH] to

furnish nine fractions [Fr. 7-1 (1.18 g), Fr. 7-2 (1.48 g), Fr. 7-3 (1.44 g), Fr. 7-4 (3.00 g), Fr. 7-5 (2.58 g), Fr. 7-6 (1.08 g), Fr. 7-7 (3.20 g), Fr. 7-8 (3.34 g), and Fr. 7-9 (6.88 g)]. Fraction 7-2 (1.48 g) was separated by HPLC [MeOH–H_2O (40 : 60, v/v)] to give 3-methoxy-4-hydroxyphenylethanol (51.9 mg, 0.0021%). Fraction 7-4 (2.76 g) was separated by normal-phase silica gel column chromatography [276 g, $CHCl_3$: MeOH : H_2O (50 : 3 : 1→30 : 3 : 1→10 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] to give seven fractions {Fr. 7-4-1 (99 mg), Fr. 7-4-2 (185 mg), Fr. 7-4-3 (52 mg), Fr. 7-4-4 (513 mg), Fr. 7-4-5 [=arctiin (**22**, 1359 mg, 0.054%)], Fr. 7-4-6 (465 mg), and Fr. 7-4-7 (45 mg)}. Fraction 7-4-1 (99 mg) was separated by HPLC [CH_3CN–H_2O (25 : 75, v/v)] to give (+)-pinoresinol (**16**, 8.9 mg, 0.0004%) and (+)-syringaresinol (**18**, 10.8 mg, 0.0004%). Fraction 7-4-4 (513 mg) was purified by HPLC [MeOH–H_2O (42 : 58, v/v)] to furnish azelaic acid (59.3 mg, 0.0024%) and (−)-secoisolariciresinol (**20**, 16.8 mg, 0.0007%). Fraction 7-5 (2.58 g) was separated by normal-phase silica gel column chromatography [250 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1→7 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] to give eight fractions {Fr. 7-5-1 [=(−)-arctigenin (**21**, 939 mg, 0.038%)], Fr. 7-5-2 (385 mg), Fr. 7-5-3 (122 mg), Fr. 7-5-4 (108 mg), Fr. 7-5-5 (229 mg), Fr. 7-5-6 (340 mg), Fr. 7-5-7 (149 mg), and Fr. 7-5-8 (168 mg)}. Fraction 7-5-4 (108 mg) was separated by HPLC [MeOH–H_2O (60 : 40, v/v)] to give luteolin (**7**, 32.0 mg, 0.0013%). Fraction 7-5-6 (340 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give quercetin (**13**, 150 mg, 0.0060%). Fraction 7-6 (1.08 g) was separated by HPLC [MeOH–H_2O (60 : 40, v/v)] to give apigenin (**3**, 53.7 mg, 0.0021%), and **7** (140 mg, 0.0056%).

The *n*-BuOH-soluble fraction (37 g) was subjected to normal-phase silica gel column chromatography [1.1 kg, $CHCl_3$–MeOH–H_2O (15 : 3 : 1→10 : 3 : 1→7 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] to furnish nine fractions [Fr. 1 (0.27 g), Fr. 2 (0.31 g), Fr. 3 (1.74 g), Fr. 4 (1.38 g), Fr. 5 (5.64 g), Fr. 6 (4.18 g), Fr. 7 (8.57 g), Fr. 8 (13.53 g), and Fr. 9 (1.21 g)]. Fraction 3 (1.74 g) was subjected to reversed-phase silica gel column chromatography [55 g, MeOH–H_2O (30 : 70→50 : 50, v/v)→MeOH] to provide five fractions [Fr. 3-1 (0.51 g), 3-2 (0.05 g), 3-3 (**22**, 983 mg, 0.062%), 3-4 (0.02 g), 3-5 (0.11 g), and Fr. 3-6 (0.06 g)]. Fr. 3-2 (0.05 g) was purified by HPLC [MeOH–H_2O (40 : 60, v/v)] to give (+)-syringaresinol *O*-β-D-glucopyranoside (**19**, 16.7 mg, 0.0011%). Fr. 3-5 (0.11 g) was purified by HPLC [MeOH–H_2O (50 : 50, v/v)] to give sitosterol 3β-*O*-β-D-glucopyranoside (72.5 mg, 0.0046%). Fraction 4 (1.38 g) was subjected to reversed-phase silica gel column chromatography [41 g, MeOH–H_2O (30 : 70→50 : 50, v/v)→MeOH] to furnish three fractions [Fr. 4-1 (0.92 g), 4-2 (0.36 g), and 4-3 (0.10 g)]. Fraction 4-1 (0.92 g) was further purified by HPLC [MeOH–H_2O (10 : 90, 25 : 75, or 30 : 70, v/v)] to give saussureosides A (**1**, 12.0 mg, 0.0007%) and B (**2**, 59.8 mg, 0.0038%), (+)-pinoresinol *O*-β-D-glucopyranoside (**17**, 22.9 mg, 0.0026%), junipediol A (17.0 mg, 0.0011%), benzyl *O*-β-D-glucopyranoside (44.4 mg, 0.0028%), *p*-anisyl *O*-β-D-glucopyranoside (9.1 mg, 0.0006%), phenethyl *O*-β-D-glucopyranoside (11.5 mg, 0.0007%), (*Z*)-3-hexenyl *O*-β-D-glucopyranoside (16.0 mg, 0.0010%), and thymidine (41.0 mg, 0.0026%). Fraction 5 (5.64 g) was subjected to reversed-phase silica gel column chromatography [170 g, MeOH–H_2O (20 : 80→50 : 50, v/v)→MeOH] to provide seven fractions [Fr. 5-1 (2.31 g), Fr. 5-2 (0.43 g), Fr. 5-3 (0.40 g), Fr. 5-4 (0.39 g), Fr. 5-5 (0.34 g), Fr. 5-6 (1.38 g), and Fr. 5-7 (0.32 g)]. Fraction 5-2 (80 mg) was purified by HPLC [MeOH–H_2O (30 : 70, v/v)] to give syringin (22.0 mg, 0.0037%). Fraction 5-5 (340 mg) was purified by HPLC [MeOH–H_2O (45 : 65, v/v)] to furnish quercetin 3-*O*-β-D-glucopyranoside (**14**, 60.2 mg, 0.0038%). Fraction 5-6 (374 mg) was separated by HPLC [MeOH–H_2O (40 : 60, v/v)] to give apigenin 7-*O*-β-D-glucopyranoside (**4**, 7.0 mg, 0.0016%), apigenin 4′-*O*-β-D-glucopyranoside (**5**, 38.4 mg, 0.0089%), luteolin 7-*O*-β-D-glucopyranoside (**8**, 7.6 mg, 0.0018%), luteolin 4′-*O*-β-D-glucopyranoside (**9**, 120.4 mg, 0.028%), chrysoeriol 7-*O*-β-D-glucopyranoside (**11**, 14.6 mg, 0.0034%), and **14** (69.5 mg, 0.016%). Fraction 6 (4.18 g) was subjected to reversed-phase silica gel column chromatography [140 g, MeOH–H_2O (30 : 70→50 : 50→70 : 30, v/v)→MeOH] to provide four fractions [Fr. 6-1 (1.50 g), Fr. 6-2 (2.30 g), Fr. 6-3 (0.26 g), and Fr. 6-4 (0.08 g)]. Fraction 6-1 (0.50 g) was separated by HPLC [MeOH–H_2O (17 : 83, v/v)] to give L-phenylalanin (9.7 mg, 0.0018%). Fraction 6-2 (0.50 g) was separated by HPLC [MeOH–H_2O (40 : 60, v/v)] to give **6** (14.6 mg, 0.0054%), **8** (61.7 mg, 0.023%), **9** (39.0 mg, 0.014%), chrysoeriol 7-*O*-rutinoside (**12**, 8.1 mg, 0.0035%), **14** (160 mg, 0.059%), and isorhamnetin 3-*O*-rutinoside (**15**, 9.6 mg, 0.0035%). Fraction 7 (8.57 g) was subjected to reversed-phase silica gel column chromatography [260 g, MeOH–H_2O (30 : 70→50 : 50→70 : 30, v/v)→MeOH] to provide five fractions [Fr. 7-1 (3.51 g), Fr. 7-2 (2.96 g), Fr. 7-3 (1.14 g), Fr. 7-4 (0.35 g), and Fr. 7-5 (0.36 g)]. Fraction 7-2 (350 mg) was separated by HPLC [MeOH–H_2O (40 : 60, v/v)] to afford **7** (18.0 mg, 0.0096%), **8** (18.0 mg, 0.0096%), luteolin 7-*O*-rutinoside (**10**, 42.3 mg, 0.023%), and 5-*O*-caffeoylquinic acid methyl ester (**25**, 27.0 mg, 0.015%). Fraction 8 (15.53 g) was subjected to reversed-phase silica gel column chromatography [470 g, MeOH–H_2O (10 : 90→25 : 75→40 : 60→50 : 50, v/v)→MeOH] to provide four fractions [Fr. 8-1 (9.16 g), Fr. 8-2 (2.65 g), Fr. 8-3 (1.15 g), and Fr. 8-4 (1.85 g)]. Fraction 8-2 (600 mg) was separated by HPLC [MeOH–H_2O (33 : 67, v/v)] to furnish 3-*O*-caffeoylquinic acid methyl ester (**23**, 4.0 mg, 0.0011%), 4-*O*-caffeoylquinic acid methyl ester (**24**, 4.2 mg, 0.0012%), and **25** (15.4 mg, 0.0043%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR, MS] with reported values[18—36,38—45] or commercial samples.[37]

Saussureoside A (**1**): A white powder, $[\alpha]_D^{24}$ −45.0° (*c*=0.80, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{30}O_9Na$ $(M+Na)^+$ 425.1788; Found 425.1783. CD [MeOH, nm ($\Delta\varepsilon$)]: 229 (−4.36). UV [MeOH, nm (log ε)]: 229 (4.07). IR (KBr): 3410, 2932, 1684, 1671, 1260, 1078, 756 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: given in Table 2. ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 3. Positive-ion FAB-MS: *m*/*z* 425 $(M+Na)^+$.

Saussureoside B (**2**): A white powder, $[\alpha]_D^{26}$ −39.5° (*c*=0.98, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{30}O_8Na$ $(M+Na)^+$ 409.1838; Found 409.1843. CD [MeOH, nm ($\Delta\varepsilon$)]: 227 (+3.21), 279 (+3.16), 315 (−3.06). UV [MeOH, nm (log ε)]: 227 (3.89), 279 (3.78). IR (KBr): 3432, 2932, 1671, 1655, 1364, 1262, 1076 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: given in Table 2. ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 3. Positive-ion FAB-MS: *m*/*z* 409 $(M+Na)^+$.

Acid Hydrolysis of 1 and 2 A solution of **1** and **2** (2.0 mg of each) in 1 M HCl (0.5 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo)]; mobile phase, CH_3CN–H_2O (75 : 25, v/v); flow rate 0.8 ml/min; column temperature, room temperature. Identification of D-glucose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with those of an authentic sample. t_R: 12.3 min (positive optical rotation).

Enzymatic Hydrolysis of 1 and 2 with β-Glucosidase A solution of **1** (8.0 mg, 0.020 mmol) in H_2O (1.0 ml) was treated with β-glucosidase (8.0 mg, from Almond, Oriental Yeast Co., Ltd., Japan) and the solution was stirred at 37 °C for 4 d. After EtOH was added to the reaction mixture, the solvent was removed under reduced pressure and the residue was purified by HPLC [MeOH–H_2O (40 : 60, v/v)] to furnish **1a** (3.4 mg, 71%). Through a similar procedure, **2a** (4.8 mg, 75%) was prepared from **2** (9.4 mg, 0.024 mol).

1a: A white powder, $[\alpha]_D^{18}$ −64.6° (*c*=0.10, MeOH). High-resolution EI-MS: Calcd for $C_{13}H_{20}O_4$ (M^+) 240.1361; Found 240.1354. CD [MeOH, nm ($\Delta\varepsilon$)]: 229 (+3.92). UV [MeOH, nm (log ε)]: 229 (3.92), UV [EtOH, nm (log ε)]: 232 (4.05). IR (KBr): 3432, 2924, 1682, 1662, 1258, 754 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.99, 1.17, 1.31, 2.28 (each 3H, all s, 12, 13, 11, 10-H_3), [1.30 (1H, dd, *J*=3.4, 12.5 Hz), 1.60 (1H, dd, *J*=12.5, 12.5 Hz), 2-H_2], 3.96 (1H, ddd, *J*=3.4, 3.4, 12.5 Hz, 3-H), 4.02 (1H, d, *J*=3.4 Hz, 4-H), 6.27, 7.01 (1H each, both d, *J*=15.9 Hz, 8, 7-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 3. EI-MS *m*/*z* (%): 240 (M^+, 2), 179 (3), 151 (3), 123 (100).

2a: A white powder, $[\alpha]_D^{25}$ −70.7° (*c*=0.25, MeOH). High-resolution EI-MS: Calcd for $C_{13}H_{20}O_3$ (M^+) 224.1412; Found 224.1415. CD [MeOH, nm ($\Delta\varepsilon$)]: 225 (+3.21), 274 (+3.28), 312 (−2.94). UV [MeOH, nm (log ε)]: 220 (3.87), 281 (3.82). IR (KBr): 3410, 2934, 1674, 1609, 1363, 1258, 1063 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.05, 1.12, 2.07, 2.32 (each 3H, all s, 13, 12, 11, 10-H_3), [1.78 (1H, dd, *J*=3.4, 12.5 Hz), 2.07 (1H, dd, *J*=12.5, 12.5 Hz), 2-H_2], 4.18 (1H, ddd, *J*=3.4, 3.4, 12.5 Hz, 3-H), 4.30 (1H, d, *J*=3.4 Hz, 4-H), 6.32, 7.39 (1H each, both d, *J*=16.5 Hz, 8, 7-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 3. EI-MS *m*/*z* (%): 224 (M^+, 2), 206 (16), 191 (100).

Preparation of the (*R*)-MTPA Esters (1b, 2b) and (*S*)-MTPA Esters (1c, 2c) from 1a and 2a A solution of **1a** or **2a** (0.7 mg each) in CH_2Cl_2 (1.0 ml) was treated with (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid [(*R*)-MTPA, 3.4 mg] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl, 2.9 mg) and 4-dimethylaminopyridine (4-DMAP, 1.1 mg), and the mixture was stirred at room temperature for 10 h. After cooling, the reaction mixture was poured into ice-water and the whole reaction mixture was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and

brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was separated by HPLC [MeOH–H_2O (70 : 30, v/v)] to give **1b** (0.7 mg, 53%) or **2b** (0.7 mg, 51%), respectively. Using a similar procedure, (*S*)-MTPA esters [**1c** (1.0 mg, 53%) or **2c** (1.2 mg, 51%)] were obtained from **1a** or **2a** (1.0 mg each), respectively, using (*S*)-MTPA (4.9 mg), EDC·HCl (4.2 mg), and 4-DMAP (1.6 mg).

1b: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.06, 1.16, 1.26, 2.27 (each 3H, all s, 12, 13, 11, 10-H_3), [1.44 (1H, dd, J=3.4, 12.5 Hz), 1.91 (1H, dd, J=12.5, 12.5 Hz), 2-H_2], 3.54 (3H, s, –OCH_3), 4.11 (1H, d, J=3.4 Hz, 4-H), 5.28 (1H, ddd, J=3.4, 3.4, 12.5 Hz, 3-H), 6.27, 6.96 (1H each, both d, J=16.5 Hz, 8, 7-H), [7.40—7.42 (3H, m), 7.48—7.50 (2H, m), Ph-H].

1c: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.06, 1.15, 1.30, 2.29 (each 3H, all s, 12, 13, 11, 10-H_3), [1.44 (1H, dd, J=3.4, 12.5 Hz), 1.83 (1H, dd, J=12.5, 12.5 Hz), 2-H_2], 3.52 (3H, s, –OCH_3), 4.21 (1H, d, J=3.4 Hz, 4-H), 5.28 (1H, ddd, J=3.4, 3.4, 12.5 Hz, 3-H), 6.29, 6.98 (1H each, both d, J=16.5 Hz, 8, 7-H), [7.42—7.44 (3H, m), 7.50—7.51 (2H, m), Ph-H].

2b: ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.05, 1.15, 1.99, 2.33 (each 3H, all s, 13, 12, 11, 10-H_3), [1.91 (1H, dd, J=3.4, 12.5 Hz), 2.45 (1H, dd, J=12.5, 12.5 Hz), 2-H_2], 3.74 (3H, s, –OCH_3), 4.47 (1H, d, J=3.4 Hz, 4-H), 5.50 (1H, ddd, J=3.4, 3.4, 12.5 Hz, 3-H), 6.32, 7.35 (1H each, both d, J=16.5 Hz, 8, 7-H), [7.42—7.47 (3H, m), 7.94 (2H, d, J=7.6 Hz), Ph-H].

2c: ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.96, 1.13, 2.01, 2.33 (each 3H, all s, 13, 12, 11, 10-H_3), [1.75 (1H, dd, J=3.4, 12.5 Hz), 2.38 (1H, dd, J=12.5, 12.5 Hz), 2-H_2], 3.81 (3H, s, –OCH_3), 4.63 (1H, d, J=3.4 Hz, 4-H), 5.52 (1H, ddd, J=3.4, 3.4, 12.5 Hz, 3-H), 6.30, 7.32 (1H each, both d, J=16.5 Hz, 8, 7-H), [7.40—7.47 (3H, m), 8.00 (2H, d, J=7.6 Hz), Ph-H].

Bioassay. Inhibitory Effect on Aldose Reductase Activity Aldose reductase activity was assayed by the method described previously.[52—57] The supernatant fluid of rat lens homogenate was used as a crude enzyme. The incubation mixture 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 100 μl of enzyme fraction, with or without 25 μl of sample solution, in a total volume of 0.5 ml. The reaction was initiated by the addition of NADPH at 30 °C. After 30 min, the reaction was stopped by the addition of 150 μl of 0.5 M HCl. Then, 0.5 ml 6 M NaOH containing 10 mM imidazole was added, and the solution was heated at 60 °C for 20 min to convert NADP to a fluorescent product. Fluorescence was measured using a fluorophotometer (Luminescence Spectrometer LS50B, Perkin Elmer, U.K.) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

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