Structures of New Friedelane-Type Triterpenes and Eudesmane-Type Sesquiterpene and Aldose Reductase Inhibitors from *Salacia chinensis*

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Received April 9, 2003

Three new friedelane-type triterpenes named salasones A (**1**), B (**2**), and C (**3**), a new norfriedelane-type triterpene, salaquinone A (**4**), and a new acylated eudesmane-type sesquiterpene, salasol A (**5**), were isolated from the 80% aqueous methanolic extract of the stems of *Salacia chinensis* collected in Thailand. Their stereostructures were elucidated on the basis of chemical and physicochemical evidence. In addition, six constituents, 3β,22β-dihydroxyolean-12-en-29-oic acid, tingenone, tingenine B, regeol A, triptocalline A, and mangiferin, were found to show an inhibitory effect on rat lens aldose reductase.

Salacia chinensis L. (syn. *S. prinoides,* Hippocrateaceae)[1] is widely distributed in Thailand, Myanmar, and India. The stems of *S. chinensis* have been extensively used for carminative, emmenagogue, blood tonic, cardiotonic, anti-inflammatory, and antidiabetic purposes and the treatment of rheumatism, leukorrhea, and stimulated lochial excretion. Previously, we reported that the extracts of *S. reticulata* collected in Sri Lanka[2−4] and *S. oblonga* collected in India[5] were found to show hypoglycemic effects in oral sucrose- and maltose-loaded rats and α-glucosidase inhibitory activities against sucrase, maltase, and isomaltase. By bioassay-guided separation, we isolated and elucidated the stereostructures of potent α-glucosidase inhibitors, salacinol and kotalanol, from *S. reticulata* and *S. oblonga*.[2−5] In addition, an azacyclic analogue of salacinol with α-glucosidase inhibitory activities was synthesized.[6] The extract of the stems of *S. reticulata* and its components were found to show rat lens aldose reductase inhibitory activity,[7] hepatoprotective effect on CCl_4-induced liver injury,[8] antioxidative activity,[8] and antiobese activity.[9] Moreover, we described the HPLC quantitative analytical method for mangiferin, which is a common principal constituent of *Salacia* species.[7] In our continuing study of *Salacia* species, we found that the 80% aqueous methanolic extract of *S. chinensis* collected in Thailand showed hypoglycemic effects, gastroprotective effects, α-glucosidase and aldose reductase inhibitory activities, nitric oxide production inhibitory effects, and antioxidative activity.[10] From the 80% aqueous methanolic extract, three new friedelane-type triterpenes, salasones A (**1**), B (**2**), and C (**3**), a new norfriedelane-type triterpene, salaquinone A (**4**), and a new acylated eudesmane-type sesquiterpene, salasol A (**5**), were isolated together with 28 known compounds. This paper deals with the isolation and structure elucidation of five new constituents (**1**−**5**) and inhibitory effects of the principal constituents on rat lens aldose reductase.

Results and Discussion

The stems of *S. chinensis* (collected in Phiphun District, Nakhon Si Thammarat Province, Thailand) were finely cut and extracted with 80% aqueous methanol under reflux. The 80% aqueous methanolic extract was partitioned into a mixture of ethyl acetate (EtOAc) and water to furnish the EtOAc-soluble fraction and H_2O-soluble fraction. The EtOAc-soluble fraction was separated by silica gel and octadecyl silanized silica gel (ODS) column chromatography and finally HPLC (ODS) to give salasones A (**1**, 0.0044% from the natural medicine), B (**2**, 0.0005%), and C (**3**, 0.0013%), salaquinone A (**4**, 0.0006%), and salasol A (**5**, 0.0032%) together with six friedelane-type triterpenes, maytenoic acid[11] (0.0016%), friedelan-3-on-29-ol[12,13] (**7**, 0.0014%), 15α-hydroxyfridelan-3-one[14] (0.017%), wilforic acid C[11] (0.0006%), salaspermic acid (0.0017%), and orthosphenic acid (0.0018%), five oleanane-type triterpenes, 3β,22β-dihydroxyolean-12-en-29-oic acid[18] (0.0014%), maytenfolic acid[18,19] (0.0055%), β-amyrin[20] (0.0027%), 22α-hydroxy-3-oxoolean-12-en-29-oic acid[18] (0.0046%), and β-amyrenone[21] (0.0009%), two ursane-type triterpenes, tripterygic acid A[22] (0.0007%) and demethylregelin[23] (0.0016%), four norfriedelane-type triterpenes, tingenone[23] (0.0005%), tingenin B (**8** = 22β-hydroxytingenone,[23−25] 0.0005%), regeol A[24] (0.0018%), and triptocalline A[25] (0.0026%), a eudesmane-type sesquiterpene, celahin C[26] (**9**, 0.0007%), a xanthone, mangiferin[7−9] (0.016%), two flavones, vitexin[27] (0.0016%) and isovitexin[27] (0.0036%), three lignanes, (+)-lyoniresinol[28] (0.0008%), (+)-isolariciresinol[29,30] (0.0025%), and (+)-8-methoxyisolariciresinol[30,31] (0.0008%), three flavan-3-ols, (−)-epigallocatechin[7−10] (0.0006%), (−)-epicatechin[7−10] (0.017%), and (+)-catechin[7−10] (0.0013%), and squalene[32] (0.014%). Salasone A (**1**) was obtained as a white powder with negative optical rotation ($[\alpha]_D^{26}$ −31.8°, $CHCl_3$). The IR spectrum of **1** showed absorption bands at 3550, 1717, and 1692 cm^{-1} ascribable to hydroxyl and carbonyl functions. In the EIMS of **1**, the molecular ion peak was observed at *m/z* 456 [M^+], and the HREIMS analysis revealed the molecular formula of **1** to be $C_{30}H_{48}O_3$. The ^1H NMR ($CDCl_3$) and ^{13}C NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[33] showed signals assignable to seven methyls [δ 0.77, 0.88, 0.97, 1.00, 1.05, 1.38 (3H each, all s, H_3-24, 27, 30, 25, 29, 28), 0.90 (3H, d, J = 6.9 Hz, H_3-23)], a methylene with an oxygen function [δ 4.16, 4.41 (1H each, both d, J = 12.1 Hz, H_2-26)], and two carbonyl groups [δ_C 211.6 (C-15), 212.4 (C-3)] together with 10 methylenes (C-1, 2, 6, 7, 11, 12, 16, 19, 21, 22), four methines (C-4, 8, 10, 18), and six quaternary carbons (C-5, 9, 13, 14, 17, 20). The proton and carbon signals in the ^1H and ^{13}C NMR spectra of **1** were superimposable on those of kokoonol (**6**),[34] except for the signals due to the 15-carbonyl group. The planar structure of **1** was elucidated on the basis of H−H COSY and HMBC experiments. Namely, the H−H COSY

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10.1021/np0301543 CCC: $25.00
Published on Web 08/29/2003

Chart 1

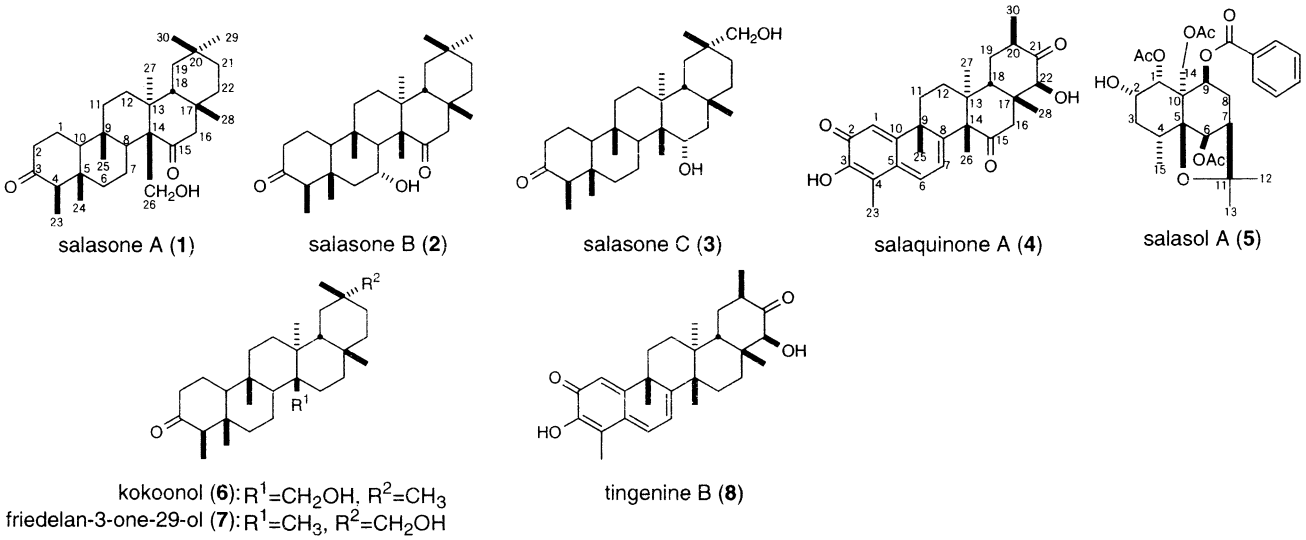

Table 1. ^{13}C NMR Data of Salasones A−C (**1**−**3**), Salaquinone A (**4**), Salasol A (**5**), and 3,4-Dideoxymaytol (**5a**)

	1[a]	**2**[a]	**3**[a]	**4**[a]		**5**[a]	**5a**[b]
C-1	22.2	21.8	22.3	119.2	C-1	74.5	74.3
C-2	41.3	41.0	41.4	178.0	C-2	68.3	69.5
C-3	212.4	211.9	212.9	145.9	C-3	32.4	35.0
C-4	58.1	58.1	58.0	117.5	C-4	33.2	35.2
C-5	41.9	42.6	41.9	128.0	C-5	89.6	93.5
C-6	40.8	51.5	41.1	132.9	C-6	78.1	77.7
C-7	21.4	66.7	19.9	124.3	C-7	48.7	52.5
C-8	45.3	49.7	53.4	157.1	C-8	34.9	35.2
C-9	37.4	38.5	37.7	42.5	C-9	69.8	73.2
C-10	59.2	58.9	59.2	163.1	C-10	53.4	55.4
C-11	34.0	34.8	35.6	31.7	C-11	82.3	83.4
C-12	30.9	29.4	31.1	28.9	C-12	30.2	31.3
C-13	42.4	43.6	40.6	43.8	C-13	25.9	27.2
C-14	59.8	54.6	44.0	58.0	C-14	65.9	66.4
C-15	211.6	220.0	74.4	209.3	C-15	18.0	20.1
C-16	54.3	54.3	48.1	47.8			
C-17	32.6	34.7	30.6	47.8	1-OAc	169.3	
C-18	44.1	44.2	40.8	43.9		20.7	
C-19	35.7	34.5	29.8	30.2	6-OAc	169.8	
C-20	28.0	27.9	33.0	39.9		21.3	
C-21	32.9	33.9	27.1	212.0	10-OAc	170.3	
C-22	39.3	38.5	38.9	78.0		21.2	
C-23	6.8	6.9	6.8	10.3			
C-24	15.0	16.0	14.5		9-OBz		
C-25	17.6	17.8	17.9	39.4	C-1′	129.0	
C-26	19.9	20.0	18.7	21.5	C-2′,6′	129.9	
C-27	60.5	15.0	14.1	24.4	C-3′,5′	128.1	
C-28	32.5	31.8	32.6	24.8	C-4′	133.2	
C-29	34.6	33.5	74.8		C-7′	165.0	
C-30	31.5	32.8	25.4	15.0			

[a] Measured in $CDCl_3$. [b] Measured in CD_3OD at 125 MHz.

experiment on **1** indicated the presence of six partial structures shown in bold lines in Figure 1 (C-10−1−2, C-4−23, C-6−8, C-11−12, C-18−19, and C-21−22). In the HMBC experiment, long-range correlations were observed between the following proton and carbon pairs (H-4 and C-2, 3, 5, 23; H_2-16 and C-14, 15; H_3-23 and C-3−5; H_3-24 and C-4−6, 10; H_3-25 and C-8−11; H_3-26 and C-8, 13−15; H_3-27 and C-12−14, 18; H_3-28 and C-16−18, 22; H_3-29 and C-19−21, 30; H_3-30 and C-19−21, 29) as shown in Figure 1. The above evidence led us to clarify the connectivities of the quaternary carbons and the positions of the carbonyl and hydroxyl groups in a friedelane skeleton.

Finally, the stereostructure of **1** was confirmed by NOESY experiment, which showed NOE correlations between the following proton pairs (H-18 and H_3-28, 30; H_3-23 and H_3-24; H_3-24 and H_3-25; H_3-25 and H_2-26; H_2-26 and H_3-28; H_3-28 and H_3-30). Consequently, the stereostructure of **1** was determined as 26-hydroxyfriedelane-3,15-dione.

Salasone B (**2**) was obtained as a white powder with negative optical rotation ($[\alpha]_D^{27}$ −7.6°, $CHCl_3$). The IR spectrum of **2** showed absorption bands at 3459, 1717, and 1678 cm^{-1} ascribable to hydroxyl and carbonyl functions. The molecular formula $C_{30}H_{48}O_3$ of **2**, which was the same as that of **1**, was determined from the molecular ion peaks at *m/z* 456 $[M]^+$ in EIMS and by HREIMS measurements. The ^1H NMR ($CDCl_3$) and ^{13}C NMR (Table 1) spectra[33] of **2** showed signals assignable to eight methyls [δ 0.81, 0.87, 1.02, 1.37, 1.44 (3H each, all s, H_3-24, 27, 29, 28, 26), 0.93 (3H, d, J = 7.0 Hz, H_3-23), 0.97 (6H, s, H_3-25, 30)], a methine bearing an oxygen function [δ 3.71 (1H, ddd, J = 3.6, 10.8, 10.8 Hz, H-7)], and two carbonyl groups [δ_C 211.9 (C-3), 220.0 (C-15)] together with nine methylenes (C-1, 2, 6, 11, 12, 16, 19, 21, 22), four methines (C-4, 8, 10, 18), and six quaternary carbons (C-5, 9, 13, 14, 17, 20). The H−H COSY experiment on **2** showed the presence of partial structures similar to those of **1**, except for the 7-hydroxyl group (Figure 1). In the HMBC experiment of **2**, long-range correlations were observed following proton and carbon pairs (H-4 and C-2, 3, 5, 23; H_3-23 and C-3−5; H_3-24 and C-4−6, 10; H_3-25 and C-8−11; H_3-26 and C-8, 13−15; H_3-27 and C-12−14, 18; H_3-28 and C-16−18, 22; H_3-29 and C-19−21, 30; H_3-30 and C-19−21, 29). The above evidence indicated the positions of the 3- and 15-carbonyl and 7-hydroxyl functions in the friedelane skeleton. Furthermore, the stereostructure of the 7α-hydroxyl group in **2** was determined by NOESY experiment, which showed NOE correlations between the following proton pairs (H-7 and H_3-24−26; H-18 and H_3-28, 30; H_3-23 and H_3-24; H_3-24 and H_3-25; H_3-25 and H_3-26; H_3-26 and H_3-28; H_3-28 and H_3-30) as shown in Figure 1. Finally, by comparison of the ^1H and ^{13}C NMR data for **2** with those for **1** and related friedelane-type triterpenes,[34] the stereostructure of **2** was confirmed as 7α-hydroxyfriedelane-3,15-dione.

Salasone C (**3**) was also isolated as a white powder with negative optical rotation ($[\alpha]_D^{25}$ −21.9°, $CHCl_3$). The EIMS of **3** showed a molecular ion peak at *m/z* 458 $[M^+]$, and the HREIMS analysis revealed the molecular formula of **3** to be $C_{30}H_{50}O_3$. The IR spectra showed absorption bands assignable to hydroxyl and carbonyl groups (3453 and 1716 cm^{-1}). The ^1H NMR ($CDCl_3$) and ^{13}C NMR (Table 1)

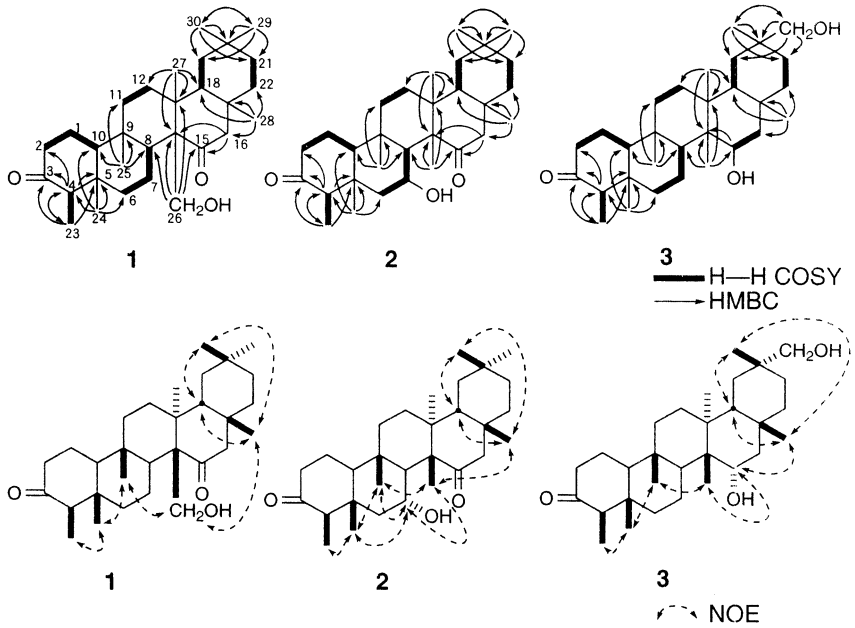

Figure 1. H−H COSY, HMBC, and NOE correlations of **1**−**3**.

spectra[33] of **3** showed signals assignable to seven methyls [δ 0.73, 0.89, 1.01, 1.04, 1.08, 1.33 (3H each, all s, H_3-24, 25, 27, 30, 26, 28), 0.88 (3H, d, J = 6.8 Hz, H_3-23)], a methylene [δ 3.23, 3.27 (1H each, both d, J = 11.4 Hz, H_2-29)] and a methine bearing an oxygen function [δ 3.74 (1H, d, J = 7.9 Hz, H-15)], and a carbonyl group [δ_C 212.9 (C-3)] together with 10 methylenes, four methines, and six quaternary carbons. The proton and carbon signals in the ^{1}H and ^{13}C NMR spectra of **3** were very similar to those of friedelane-3-one-29-ol (**7**),[12,13] except for the signals due to the 15-hydroxyl group. The positions of hydroxyl and carbonyl groups of **3** were elucidated on the basis of HMBC experiments as shown in Figure 1. Furthermore, the NOESY experiment on **3** showed NOE correlations between the following proton pairs (H-15 and H_3-26, 28; H-18 and H_3-28, 30; H_3-23 and H_3-24; H_3-24 and H_3-25; H_3-25 and H_3-26; H_3-28 and H_3-30), so that the stereostructure of **3** including the 15α-hydroxyl group was characterized. These findings led us to confirm the stereostructure of salasone C as 15α,29-dihydroxyfriedelan-3-one (**3**).

Salaquinone A (**4**) was obtained as an amorphous powder with positive optical rotation ([α]$_D^{24}$ +95.4° $CHCl_3$). The IR spectrum of **4** showed absorption bands at 3548 and 1717 cm^{-1} ascribable to hydroxyl and carbonyl functions. In the UV spectrum of **4**, absorption maxima were observed at 249 nm (log ϵ 3.8) and 416 nm (3.9). The molecular formula $C_{28}H_{34}O_5$ of **4** was characterized from the EIMS and by HREIMS measurement. The ^{1}H NMR ($CDCl_3$) and ^{13}C NMR (Table 1) spectra[33] of **4** showed signals assignable to six methyls [δ 1.05, 1.05, 1.50, 1.71, 2.22 (3H each, all s, H_3-27, 28, 25, 26, 23), 1.15 (3H, d, J = 6.6 Hz, H_3-30)], a methine bearing an oxygen function [δ 4.43 (1H, d, J = 2.9 Hz, H-22)], three olefins [δ 6.49 (1H, s, H-1), 6.97, 7.02 (1H each, both d, J = 7.2 Hz, H-7, 6)], and three carbonyl groups [δ_C 178.0 (C-2), 209.3 (C-15), 212.0 (C-21)] together with four methylenes (C-11, 12, 16, 19), two methines (C-18, 20), and nine quaternary carbons (C-3, 4, 5, 8, 9, 10, 13, 14, 17). The proton and carbon signals in the ^{1}H and ^{13}C NMR data of **4** resembled those of tingenine B (**8**),[23−25] except for the signals due to the 15-carbonyl group. As shown in Figure 2, the H−H COSY experiment of **4** indicated the presence of three partial structures shown in bold lines (C-6−7, C-11−12, C-18−20−30). In the HMBC

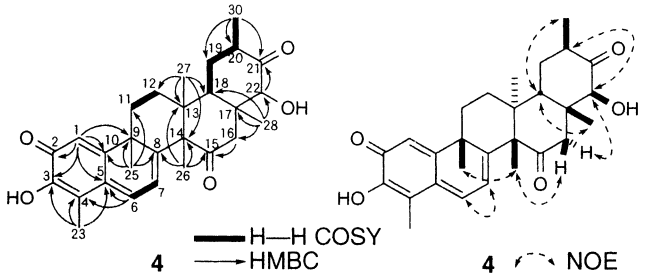

Figure 2. H−H COSY, HMBC, and NOE correlations of **4**.

experiment of **4**, long-range correlations were observed between the following proton and carbon pairs (H-1 and C-2, 3, 5, 9, 10; H-6 and C-4, 5; H_2-16 and C-15; H-22 and C-21; H_3-23 and C-3−5; H_3-25 and C-8−11; H_3-26 and C-8, 13−15; H_3-27 and C-12−14, 18; H_3-28 and C-16−18, 22; H_3-30 and C-19−21), so that the positions of the carbonyl and olefin functions and quaternary carbons of **4** were clarified as shown in Figure 2. Finally, the stereostructure of **4** including the 22β-hydroxyl group was confirmed by NOESY experiment. The NOE correlations of **4** were observed between the following proton pairs (H-6 and H-7; Hβ-16 and H_3-26; Hα-16 and H-22; H-18 and H_3-28, 30; H-20 and H-22; H_3-25 and H_3-26). Consequently, the stereostructure of salaquinone A was formulated as **4**.

Salasol A (**5**) was isolated as a white powder with positive optical rotation ([α]$_D^{24}$ +42.3°, $CHCl_3$). The EIMS of **5** showed a molecular ion peak [M$^+$] at m/z 532 in addition to a desacetylated fragment ion peak at m/z 490 [base peak]. The molecular formula $C_{28}H_{36}O_{10}$ of **5** was determined from the molecular ion peak observed in the EIMS and by HREIMS measurement. The IR spectrum of **5** showed absorption bands at 3539, 1752, 1726, 1370, 1279, and 1108 cm^{-1} ascribable to the hydroxyl, carbonyl, and aromatic functions. In the UV spectrum of **5**, absorption maxima were observed at 232 nm (log ϵ 3.4) and 275 nm (2.3), suggestive of a benzoyl group. The ^{1}H NMR ($CDCl_3$) and ^{13}C NMR (Table 1)[33] spectra of **5** showed signals assignable to three methyls [δ 1.24 (3H, d, J = 7.4 Hz, H_3-15), 1.41, 1.44 (3H each, both s, H_3-13, 12)], three acetyl groups [δ 1.63, 2.10, 2.23 (3H each, all s, Ac-1, 6, 14)], a methylene and four methine bearing an oxygen function

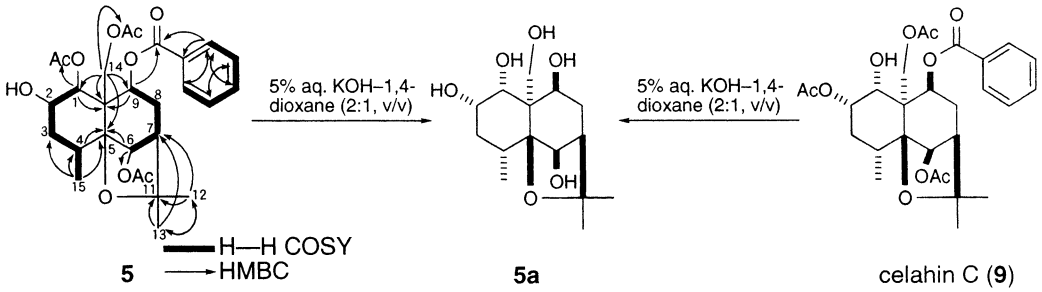

Figure 3. H−H COSY and HMBC correlations of **5**.

Table 2. Inhibitory Effects of Chemical Constituents from *S. chinensis* on Rat Lens Aldose Reductase

	IC_{50} (μM)
Friedelane-Type Triterpenes	
maytenoic acid	>100 (44)[b]
friedelane-3-on-29-ol (**7**)	98
15α-hydroxyfriedelan-3-one	>100 (8)[b]
wilfolic acid C	ca. 100 (48)[b]
salaspermic acid	>100 (2)[b]
orthosphenic acid	>100 (19)[b]
Oleanane-Type Triterpenes	
3β,22β-dihydroxyolean-12-en-29-oic acid	26
maytenfolic acid	72
β-amyrin	>100 (6)[b]
22α-hydroxy-3-oxoolean-12-en-29-oic acid	>100 (25)[b]
β-amyrenone	>100 (3)[b]
Ursane-Type Triterpenes	
tripterygic acid A	>100 (27)[b]
demethylregelin	>100 (26)[b]
Norfriedelane-Type Triterpenes	
tingenone	13
tingenin B (**8**)	7.0
regeol A	30
triptocalline A	14
Eudesmane-Type Sesquiterpene	
celahin C (**9**)	95
Others	
mangiferin	3.2
(+)-lyoniresinol	>100 (10)[b]
(+)-isolariciresinol	>100 (36)[b]
(+)-8-methoxyisolariciresinol	>100 (24)[b]
(−)-epigallocatechin	>30 (19)[a]
(−)-epicatechin	>30 (41)[a]
(+)-catechin	>30 (38)[a]

[a] Values in parentheses represent the inhibition (%) at 30 μM. [b] Inhibition (%) at 100 μM

[δ 4.40 (1H, ddd-like, H-2), 4.47, 5.13 (1H each, both d, J = 12.7 Hz, H_2-14), 5.40 (1H, d, J = 7.1 Hz, H-9), 5.63 (1H, d, J = 3.1 Hz, H-1), 6.00 (1H, br s, H-6)], and a benzoyl group [δ 7.43 (2H, dd, J = 7.1, 7.3 Hz), 7.57 (1H, t, J = 7.3 Hz), 8.04 (2H, d, J = 7.1 Hz)] together with two methylenes (H_2-3, 8), two methines (C-4, 7), and three quaternary carbons (C-5, 10, 11). The proton and carbon signals in ^{1}H and ^{13}C NMR spectra of **5** were superimposable on those of celahin C (**9**),[26] except for the signals of the 1- and 2-positions. The H−H COSY experiment on **5** indicated the presence of three partial structure written in bold lines, as shown in Figure 3. The alkaline hydrolysis of **5** with 5% aqueous potassium hydroxide (KOH) in 1,4-dioxane yielded 3,4-dideoxymaytol (**5a**),[35] which was also obtained by the alkaline hydrolysis of **9**. The positions of three acetyl groups and a benzoyl group in **5** were clarified by HMBC experiment. Namely, long-range correlations in the HMBC experiment were observed between the following protons and carbons of **5** (H-1 and C-10, *Ac*O-1; H-4 and C-5; H-6 and C-5, *Ac*O-6; H-9 and C-10, *Bz*O-9; H_3-12 and C-7, 11, 13; H_3-13 and C-7, 11, 12; H_2-14 and C-1, 5, 9, 10, *Ac*O-14; H_3-15 and C-3−5). Consequently, the stereostructure of **5** was elucidated as shown.

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 chronic complications of diabetes such as cataracts. Previously, we reported various flavonoids with inhibitory activities against rat lens aldose reductase from several natural medicines and medicinal food.[36] Since the stems of *S. chinensis* have been used for the treatment of diabetes, 25 constituents from the stems of *S. chinensis* were examined on rat lens aldose reductase inhibitory activity. As shown in Table 2, six compounds, 3β,22β-dihydroxyolean-12-en-29-oic acid (IC_{50} = 26 μM), maytenfolic acid (72 μM), tingenone (13 μM), tingenin B (**8**, 7.0 μM), regeol A (30 μM), triptocalline A (14 μM), and mangiferin (3.2 μM), were found to exhibit activity (Chart S1, Supporting Information).

Experimental Section

General Experimental Procedures. 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; EIMS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; ^{1}H NMR spectra, JEOL LNM-500 (500 MHz) spectrometer; ^{13}C NMR spectra, JEOL LNM-500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; HPLC, Shimadzu RID-6A refractive index detector.

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

Plant Material. The stems of *S. chinensis* L. were collected in Phiphun District, Nakhon Si Thammarat Province, Thailand, in July 2000. It was identified by one of the authors (Y.P.). A voucher of the plant is on file in our laboratory.

Extraction and Isolation. The dried stems of *S. chinensis* (5 kg) were crushed and extracted three times with 80% aqueous methanol under reflux. Evaporation of the solvent under reduced pressure provided the 80% aqueous methanolic extract (551 g, 11.0%), and it (538 g) was partitioned into the EtOAc−H_2O (1:1) mixture. Removal of the solvent under reduced pressure from the EtOAc- and water-soluble portion yielded 66.6 g (1.4%) and 471.4 g (9.6%) of residue, respectively. The EtOAc-soluble portion (58.7 g) was subjected to normal-phase silica gel column chromatography [1.8 kg, *n*-hexane−EtOAc (10:1 → 5:1 → 2:1 → 1:1, v/v) → $CHCl_3$−MeOH−H_2O (10:3:0.5, v/v) → MeOH] to give nine fractions {Fr.

1 [squalene (596 mg, 0.014%)], Fr. 2 (1.2 g), Fr. 3 (3.6 g), Fr. 4 (3.4 g), Fr. 5 (4.5 g), Fr. 6 (2.8 g), Fr. 7 (6.3 g), Fr. 8 (17.6 g), Fr. 9 (18.7 g)}. Fraction 2 (1.2 g) was purified by normal-phase silica gel column chromatography [36 g, *n*-hexane−EtOAc (50:1 → 15:1 → 5:1, v/v) → MeOH] to give β-amyrenone (39 mg, 0.0009%). Fraction 3 (3.6 g) was purified by normal-phase silica gel column chromatography [108 g, *n*-hexane−AcOEt (15:1 → 10:1, v/v) → MeOH] to give 15α-hydroxyfriedelan-3-one (590 mg, 0.014%) and β-amyrin (115 mg, 0.0027%). Fraction 4 (3.4 g) was purified by reversed-phase [102 g, MeOH−H_2O (70:30 → 90:10, v/v) → MeOH] and normal-phase silica gel column chromatography [36 g, *n*-hexane−EtOAc (50:1 → 15:1 → 5:1, v/v) → MeOH] to give 15α-hydroxyfriedelan-3-one (134 mg, 0.0032%). Fraction 5 (3 g) was further separated by HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 250 × 20 mm i.d.), MeOH−1% aqueous AcOH (95:5, v/v)] to give 10 fractions [Fr. 5-1 (146 mg), Fr. 5-2 (318 mg), Fr. 5-3 (298 mg), Fr. 5-4 (273 mg), Fr. 5-5 (238 mg), Fr. 5-6 (206 mg), Fr. 5-7 (387 mg), Fr. 5-8 (173 mg), Fr. 5-9 (164 mg), Fr. 5-10 (157 mg)]. Fraction 5-4 (273 mg) was purified by HPLC [MeOH−1% aqueous AcOH (80:20, v/v)] to give regeol A (50 mg, 0.0018%). Fraction 5-5 (238 mg) was purified by HPLC [MeOH−1% aqueous AcOH (85:15, v/v)] and normal-phase silica gel column chromatography [10 g, benzene−acetone (30:1, v/v) → MeOH] to give tingenone (13 mg, 0.0005%). Fraction 5-7 (387 mg) was purified by normal-phase silica gel column chromatography [30 g, $CHCl_3$−MeOH (100: 1, v/v) → MeOH] to give salasone A (**1**, 123 mg, 0.0044%). Fraction 5-8 (173 mg) was purified by normal-phase silica gel column chromatography [(i) 17 g, $CHCl_3$−MeOH (100:1, v/v) → MeOH, (ii) 10 g, benzene−acetone (40:1 → 20:1 → 10:1, v/v) → MeOH] to give salasone B (**2**, 14 mg, 0.0005%). Fraction 5-9 (164 mg) was purified by normal-phase silica gel column chromatography [16 g, $CHCl_3$−MeOH (100:1, v/v) → MeOH] to give friedelan-3-on-29-ol (**7**, 40 mg, 0.0014%). Fraction 5-10 (157 mg) was purified by normal-phase silica gel column chromatography [16 g, $CHCl_3$−MeOH (100:1, v/v) → MeOH] to give meytenoic acid (46 mg 0.0016%). Fraction 6 (2.8 g) was further separated by reversed-phase silica gel column chromatography [90 g, MeOH−H_2O (50:50 → 70:30 → 90:10, v/v) → MeOH] to give six fractions [Fr. 6-1 (412 mg), Fr. 6-2 (308 mg), Fr. 6-3 (321 mg), Fr. 6-4 (607 mg), Fr. 6-5 (676 mg), Fr. 6-6 (367 mg)]. Fraction 6-2 (308 mg) was purified by HPLC [MeOH−H_2O (70:30, v/v)] and normal-phase silica gel column chromatography [(i) 13 g, $CHCl_3$−MeOH (100:1, v/v) → MeOH, (ii) 7 g, $CHCl_3$−MeOH (300:1, v/v) → MeOH] to give salasol A (**5**, 103 mg, 0.0025%) and celahin C (**9**, 28 mg, 0.0007%). Fraction 6-3 (321 mg) was purified by HPLC [MeOH−H_2O (75:25, v/v)] and normal-phase silica gel column chromatography [15 g, *n*-hexane−AcOEt (5:1, v/v) → MeOH] to give tingenin B (**8**, 19 mg, 0.0005%). Fraction 6-4 (607 mg) was purified by HPLC [MeOH−H_2O (85:15, v/v)] and normal-phase silica gel column chromatography [15 g, $CHCl_3$−MeOH (100:1, v/v) → MeOH] to give salasone C (**3**, 55 mg, 0.0013%) and triptocalline A (107 mg, 0.0026%). Fraction 6-5 (676 mg) was purified by HPLC [MeOH−H_2O (95:5, v/v)] and normal-phase silica gel column chromatography [5 g, $CHCl_3$−MeOH (50:1, v/v) → MeOH] to give wilforic acid C (25 mg, 0.0006%) and salaspermic acid (30 mg, 0.0007%). Fraction 7 (6.0 g) was further separated by reversed-phase silica gel column chromatography [180 g, MeOH−H_2O (40:60 → 50:50 → 70:30 → 80:20 → 90:10, v/v) → MeOH] to give five fractions [Fr. 7-1 (1.8 g), Fr. 7-2 (1.5 g), Fr. 7-3 (1.0 g), Fr. 7-4 (703 mg), Fr. 7-5 (924 mg)]. Fraction 7-2 (1.5 g) was purified by normal-phase silica gel column chromatography [75 g, $CHCl_3$−MeOH (100:1, v/v) → MeOH] and HPLC [(i) MeOH−1% aqueous AcOH (75:25, v/v), (ii) CH_3CN−H_2O (55:45, v/v)] to give salaquinone A (**4**, 24 mg, 0.0006%) and **5** (26 mg, 0.0007%). Fraction 7-3 (1.0 g) was purified by normal-phase silica gel column chromatography [50 g, $CHCl_3$−MeOH (100:1, v/v) → MeOH] and HPLC [(i) MeOH−1% aqueous AcOH (85:15, v/v), (ii) CH_3CN−1% aqueous AcOH (65:35, v/v)] to give 3β,22β-dihydroxyolean-12-en-29-oic acid (55 mg, 0.0014%), maytenfolic acid (218 mg, 0.0055%), 22α-hydroxy-3-oxoolean-12-en-29-oic acid (183 mg, 0.0046%), tripterygic acid A (27 mg, 0.0007%), and demethylregelin (63 mg, 0.0016%). Fraction 7-4 (703 mg) was purified by normal-phase silica gel column chromatography [35 g, $CHCl_3$−MeOH (100:1, v/v) → MeOH] and HPLC [MeOH−1% aqueous AcOH (95:5, v/v)] to give salaspermic acid (39 mg, 0.0010%). Fraction 8 (17 g) was further separated by reversed-phase silica gel column chromatography [510 g, MeOH−H_2O (30:70 → 50:50 → 70:30 → 90:10, v/v) → MeOH] to give five fractions [Fr. 8-1 (3.7 g), Fr. 8-2 (854 mg), Fr. 8-3 (8.1 g), Fr. 8-4 (1.0 g), Fr. 8-5 (2.1 g)]. Fraction 8-1 (3.7 g) was purified by normal-phase silica gel column chromatography [200 g, $CHCl_3$−MeOH (15:1 → 10:1, v/v) → MeOH] and HPLC [MeOH−H_2O (30:70, v/v)] to give (−)-epigallocatechin (23 mg, 0.0006%), (−)-epicatechin (687 mg, 0.017%), and (+)-catechin (52 mg, 0.0013%). Fraction 8-2 (854 mg) was purified by normal-phase silica gel column chromatography [43 g, $CHCl_3$−MeOH (40:1 → 20:1 → 10:1, v/v) → MeOH] and HPLC [MeOH−H_2O (40:60, v/v)] to give (+)-lyoniresinol (32 mg, 0.0008%), (+)-isolariciresinol (102 mg, 0.0025%), and (+)-8-methoxyisolariciresinol (31 mg, 0.0008%). Fraction 8-4 (1.0 g) was purified by HPLC [MeOH−1% aqueous AcOH (90:10, v/v)] and normal-phase silica gel column chromatography [50 g, $CHCl_3$−MeOH−H_2O (30:3:1, lower layer, v/v/v) → MeOH] to give orthosphenic acid (74 mg, 0.0018%). Fraction 9 (16 g) was further separated by reversed-phase silica gel column chromatography [480 g, MeOH−H_2O (30:70 → 50:50 → 70:30, v/v/v) → MeOH] to give five fractions [Fr. 9-1 (1.8 g), Fr. 9-2 (3.5 g), Fr. 9-3 (4.4 g), Fr. 9-4 (3.9 g), Fr. 9-5 (1.7 g)]. Fraction 9-2 (3.5 g) was purified by normal-phase silica gel column chromatography [175 g, $CHCl_3$−MeOH−H_2O (10:3:1, lower layer → 6:4:1, v/v) → MeOH] to give mangiferin (561 mg, 0.016%). Fraction 9-3 (1.5 g) was purified by HPLC [MeOH−H_2O (40:60, v/v)] and normal-phase silica gel column chromatography [15 g, $CHCl_3$−MeOH−H_2O (7:3:1, lower layer, v/v/v) → MeOH] to give vitexin (56 mg, 0.0016%) and isovitexin (130 mg, 0.0036%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, ^{1}H NMR, ^{13}C NMR, MS) with reported values,[11−31] authentic samples,[2,4,7−10] or commercially obtained samples.[32]

Salasone A (1): white powder, $[\alpha]_D^{26}$ −31.8° (*c* 0.40, $CHCl_3$); IR (KBr) ν_{max} 3550, 2971, 1717, 1692, 1461, 1389 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.77, 0.88, 0.97, 1.00, 1.05, 1.38 (3H each, all s, H_3-24, 27, 30, 25, 29, 28), 0.90 (3H, d, J = 6.9 Hz, H_3-23), 1.92 (1H, dd-like, H-18), 2.24, 2.48 (1H each, both d, J = 19.2 Hz, H_2-16), 2.32 (1H, m, H-4), 4.16, 4.41 (1H each, both d, J = 12.1 Hz, H_2-26); ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 456 [M$^+$, 13], 426 [100]; HREIMS *m*/*z* 456.3612 (calcd for $C_{30}H_{48}O_3$ [M$^+$], 456.3603).

Salasone B (2): white powder, $[\alpha]_D^{27}$ −7.6° (*c* 0.70, $CHCl_3$); IR (KBr) ν_{max} 3459, 2924, 1717, 1678, 1458, 1393 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.81, 0.87, 1.02, 1.37, 1.44 (3H each, all s, H_3-24, 27, 29, 28, 26), 0.93 (3H, d, J = 7.0 Hz, H_3-23), 0.97 (6H, s, H_3-25, 30), 1.95 (1H, dd-like, H-18), 2.26, 2.70 (1H each, both d, J = 18.1, H_2-16), 2.31 (1H, m, H-4), 3.71 (1H, ddd, J = 3.6, 10.8, 10.8 Hz, H-7); ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 456 [M$^+$, 24], 423 [100]; HREIMS *m*/*z* 456.3595 (calcd for $C_{30}H_{48}O_3$ [M$^+$], 456.3603).

Salasone C (3): white powder, $[\alpha]_D^{25}$ −21.9° (*c* 0.80, $CHCl_3$); IR (KBr) ν_{max} 3453, 2930, 1716, 1458, 1389 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.73, 0.89, 1.01, 1.04, 1.08, 1.33 (3H each, all s, H_3-24, 25, 27, 30, 26, 28), 0.88 (3H, d, J = 6.8 Hz, H_3-23), [1.28 (1H, br d, J = ca. 16 Hz), 2.17 (1H, dd, J = 7.9, 15.8 Hz), H_2-16], 1.96 (1H, m, H-18), 2.23 (1H, m, H-4), 3.23, 3.27 (1H each, both d, J = 11.4 Hz, H_2-29), 3.74 (1H, d, J = 7.9 Hz, H-15); ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 458 [M$^+$, 8], 109 [100]; HREIMS *m*/*z* 458.3745 (calcd for $C_{30}H_{50}O_3$ [M$^+$], 458.3760).

Salaquinone A (4): amorphous powder, $[\alpha]_D^{24}$ +95.4° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 249 (3.8), 416 (3.9); IR (KBr) ν_{max} 3548, 2852, 1717, 1595, 1458, 1437, 1384 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.05, 1.05, 1.50, 1.71, 2.22 (3H each, all s, H_3-27, 28, 25, 26, 23), 1.15 (3H, d, J = 6.6 Hz, H_3-30), 2.26 (1H, m, H-18), 2.65 (1H, m, H-20), 2.75, 2.96 (2H, ABq, J = 15.8 Hz, H_2-16), 4.43 (1H, d, J = 2.9 Hz, H-22), 6.49 (1H, s, H-1), 6.97, 7.02 (1H each, both d, J = 7.2 Hz, H-7, 6); ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 450 [M$^+$, 100]; HREIMS *m*/*z* 450.2410 (calcd for $C_{28}H_{34}O_5$ [M$^+$], 450.2406).

Salasol A (5): white powder, $[\alpha]_D^{24}$ +42.3° (*c* 1.00, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 232 (3.4), 275 (2.3); IR (KBr) ν_{max} 3539, 3025, 2930, 1752, 1726, 1370, 1279, 1108, 714 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.24 (3H, d, J = 7.4 Hz, H_3-15), 1.41, 1.44 (3H each, both s, H_3-13, 12), 1.63, 2.10, 2.23 (3H each, all s, Ac-1, 6, 14), [1.86 (1H, br d, J = ca. 14 Hz), 2.33 (1H, m), H_2-3], [2.20 (1H, dd, J = 3.1, 15.2 Hz), 2.51 (1H, ddd, J = 3.1, 7.1, 15.2 Hz), H_2-8], 2.23 (1H, m, H-7), 2.35 (1H, m, H-4), 4.40 (1H, ddd-like, H-2), 4.47, 5.13 (1H each, both d, J = 12.7 Hz, H_2-14), 5.40 (1H, d, J = 7.1 Hz, H-9), 5.63 (1H, d, J = 3.1 Hz, H-1), 6.00 (1H, br s, H-6), [7.43 (2H, dd, J = 7.1, 7.3 Hz), 7.57 (1H, t, J = 7.3 Hz), 8.04 (2H, d, J = 7.1 Hz), Ph]; ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 532 [M^+, 27], 490 [100]; HREIMS *m*/*z* 532.2311 (calcd for $C_{28}H_{36}O_{10}$ [M^+], 532.2308).

Alkaline Hydrolysis of Salasol A (5) and Celahin C (9). A solution of **5** (5.0 mg) or celahin C (**9**, 6.5 mg) in 5% aqueous KOH-1,4-dioxane (2:1, v/v, 1.5 mL) was stirred at room temperature (25 °C) for 4 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form), and the resin was removed by filtration. After removal of the solvent from the filtrate in vacuo, the residue was separated by normal-phase silica gel column chromatography [500 mg, $CHCl_3$−MeOH−H_2O (30:3:1, lower layer, v/v/v)] to give 3,4-dideoxymaytol (**5a**, 2.7 mg, 95% from **5**; 3.4 mg, 92% from **9**). Compound **5a** was identified by comparison of physical data ($[\alpha]_D$, IR, ^1H NMR, MS) with reported values.[35]

Bioassay. Aldose Reductase Assay. Aldose reductase activity was assayed by the method described previously.[10,36] The supernatant fluid of rat lens homogenate was used as the crude enzyme. The incubation mixture contained 180 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 of 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, UK) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

Supporting Information Available: Structures of active constituents on aldose reductase inhibitory activity. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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NP0301543