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Antidiabetogenic constituents from several natural medicines*

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Abstract: In the course of our studies on antidiabetogenic and antidiabetic principles of natural medicines and medicinal foodstuffs, we have isolated salacinol and kotalanol with unique thiosugar sulfonium sulfate inner salt structures from the antidiabetic Ayurvedic traditional medicines, *Salacia reticulata* and *S. oblonga*. Salacinol and kotalanol showed potent inhibitory activities against intestinal α-glucosidase, and also inhibitory effects of salacinol on the increase in serum glucose levels in maltose- and sucrose-loaded rats were found to be more potent than those of acarbose. In addition, various flavonoids with potent inhibitory activities against rat lens aldose reductase such as quercitrin desmanthin-1 and guaijaverin were isolated from *Myrcia multiflora* and several natural medicines, and some structural requirements of flavonoids for aldose reductase inhibitory activity were clarified.

INTRODUCTION

Medicines that reduce postprandial hyperglycemia by suppressing the absorption of carbohydrates have been shown to be effective for prevention and treatment of non-insulin-dependent diabetes mellitus (NIDDM). Aldose reductase inhibitors (e.g., epalrestat) have been reported to improve the chronic complications of diabetes, such as peripheral neuropathy.

On the other hand, many traditional medicines are known to have preventive and therapeutic effects in diabetes and obesity, but their active components have not yet been characterized, except in a few cases. In the course of our studies of the bioactive principles of natural medicines and medicinal foodstuffs [1], many triterpene oligoglycosides with inhibitory effects on the increase in serum glucose levels in glucose-loaded rats were isolated from *Aralia elata* (roots, bark, young shoots), *Aesculus hippocastanum* (seeds), *Polygala senega* var. *latifolia* (roots), *Beta vulgaris* (leaves and roots), *Gymnema sylvestre* (leaves), *Kochia scoparia* (fruit), *Calendula officinalis* (flowers), etc. [1–4]. By examination of structural requirements for the inhibitory activity, the active saponins could be classified into the following three types: (1) olean-12-en-28-oic acid 3-monodesmoside (ex. elatosides, momordins, betavulgarosides, calendasaponins); (2) acylated polyhydroxyolean-12-ene 3-monodesmoside (escins, assamsaponins, camelliasaponins, gymnemosides); and (3) olean-12-ene 3,28-acylated bisdesmoside (senegasaponins, *Z*-senegins) [1–4]. These types of saponins were found to exhibit hypoglycemic effects on oral glucose-loaded rats mainly by decreasing the rate of gastric emptying and partly by inhibiting glucose transport at the small intestinal brush border. Investigation of the mode of action revealed that the inhibition of gastric emptying was mediated by capsaicin-sensitive sensory nerves and the central nervous system. Furthermore, on the basis of in vivo experiments using animals pretreated with various inhibitors, these saponins were suggested to suppress gastric emptying by stimulating the

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release and/or production of dopamine to act through dopamine$_2$ receptors, which in turn causes the release of prostaglandins [3,4].

As a continuing part of our screening for antidiabetic principles of natural medicines, we have found that the methanolic extracts from the roots and stems of *Salacia reticulata* and *S. oblonga* with intestinal α-glucosidase inhibitory activity strongly inhibited the increase in serum glucose level after administration of maltose or sucrose in rats. In addition, flavonoids from several medicinal herbs (e.g., the leaves of *Myrcia multiflora*, the flowers of *Chrysanthemum indicum*, and aerial parts of *Centella asiatica*) were found to show potent inhibitory activity against rat lens aldose reductase.

This paper focuses on our recent chemical and pharmacological studies of intestinal α-glucosidase inhibitors from *Salacia* species and aldose reductase inhibitors from several natural medicines.

α-GLUCOSIDASE INHIBITORS FROM *S. RETICULATA* AND *S. OBLONGA*

Postprandial hyperglycemia and hyperinsulinemia are expected to be reduced by inhibition of poly- and oligosaccharide digestion in the intestinal tract. Recently, intestinal α-glucosidase inhibitors (e.g., acarbose) have been used clinically for the treatment of NIDDM.

The Hippocrateaceae plants *S. reticulata* WIGHT ("Kotala himbutu" in Singhalese) and *S. oblonga* WALL. ("Chundan" in Tamil, "Ponkoranti" in Malayalam), large woody climbing plants in the submontane forests, are distributed in Sri Lanka and the southern region of India. The roots and stems of *S. reticulata* and the roots of *S. oblonga* have been extensively used for the treatment of rheumatism, gonorrhea, and skin diseases, and particularly as a specific remedy for the initial stages of diabetes in the Ayurvedic system of Indian traditional medicine.

The methanolic extracts of *S. reticulata* and *S. oblonga* (50–200 mg/kg, per os) strongly inhibited the increase in serum glucose level after administration of maltose or sucrose, but not glucose, in rats. Furthermore, the extracts inhibited rat intestinal maltase and sucrase in vitro; their IC_{50} values were 42 and 66 μg/ml, respectively, for maltase (substrate: maltose 37 mM), and 32 and 24 μg/ml, respectively, for sucrase (substrate: sucrose 37 mM), although even at high doses the extracts did not have any effect on experimental hyperglycemia induced by injection of alloxan in mice. These observations suggested that the traditional antidiabetic property of these natural medicines was attributable to intestinal α-glucosidase inhibitory activity, and we undertook the bioassay-guided separation using intestinal α-glucosidase inhibitory activity to elucidate the active constituents. Two potent α-glucosidase inhibitors named salacinol (**1**) and kotalanol (**2**) were isolated together with many known sugars, which were the principal components of their water-soluble fractions [5–8], and a xanthone, mangiferin (**3**) [9].

The planar structure of salacinol (**1**) was elucidated on the basis of physicochemical evidence, including the results of detailed NMR analysis. Alkaline treatment of salacinol (**1**) with sodium methoxide gave 1-deoxy-4-thio-D-arabinofuranose (**1a**), which was identical to the synthetic compound from D-xylose (Fig. 1). Finally, the stereostructure was clarified by X-ray crystallographic analysis, which showed the unique spiro-like conformation of the inner salt comprised of the 1-deoxy-4-thio-D-arabinofuranosyl sulfonium cation and 1-deoxy-D-erythrosyl-3-sulfate anion [5–7]. The structure of kotalanol (**2**) was also characterized, except for the stereostructure of the 1-deoxyheptosyl-3-sulfate anion moiety, on the basis of chemical and physicochemical evidence as shown [6]. As far as we know, 5-thiogalactopyranose was hitherto isolated from a marine sponge as the only natural thiosugar. Compounds **1** and **2** are the first examples of sulfonium-type thiosugars.

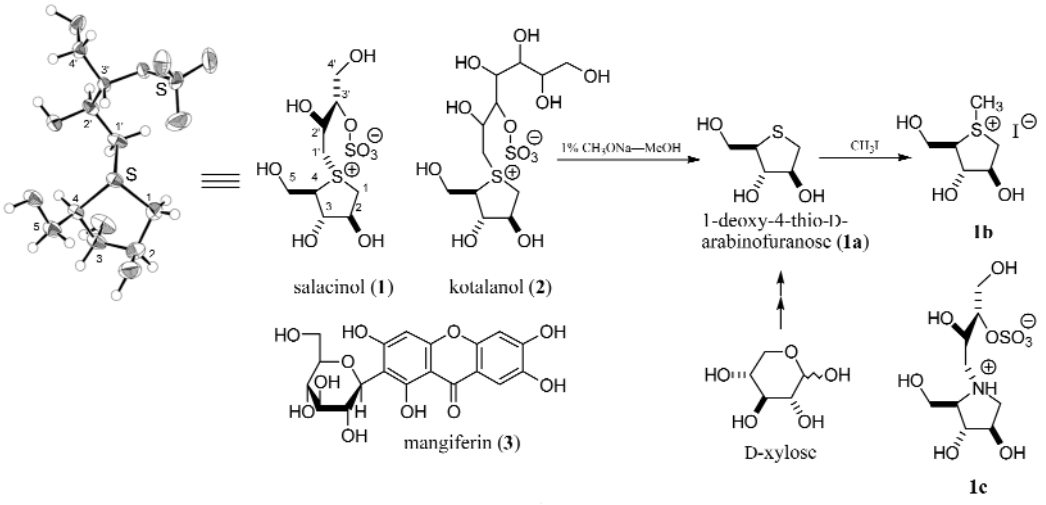


Fig. 1

α-Glucosidase inhibitory activities of salacinol (1) and kotalanol (2)

Salacinol (**1**) and kotalanol (**2**) showed inhibitory activities against small intestinal α-glucosidase in vitro. Mangiferin (**3**) inhibited both sucrase (IC_{50} = 87 μg/ml) and aldose reductase (IC_{50} = 1.4 μg/ml) [9], but other principal sugars such as dulcitol exhibited no activity. Table 1 shows IC_{50} values of **1**, **2**, and commercial medicines as α-glucosidase inhibitors, acarbose and voglibose. The maltase inhibitory activities of **1** and **2** were slightly weaker than that of acarbose and the sucrase inhibitory activities were similar to that of acarbose, while the isomaltase inhibitory activities were more potent than that of acarbose and nearly equivalent to that of voglibose.

On the other hand, 1-deoxy-4-thio-D-arabinofuranose (**1a**) lacked this activity (maltase and sucrase, IC_{50} > 400 μg/ml) and its methyl sulfonium iodide (**1b**) showed weak activity (maltase, >400 μg/ml; sucrase, 129 μg/ml). These observations indicated that the spiro-like inner salt structure was essential for potent α-glucosidase inhibitory activity. In addition, substitution of the sulfur atom in **1** with a nitrogen reduced the activity considerably (**1c**: maltase, 97 μg/ml; sucrase, 14 μg/ml) [10].

To examine the types of inhibition of maltase, sucrase, and isomaltase by **1** and **2**, small intestinal brush border membrane vesicles were incubated with increasing concentrations of maltose (3.1–37 mM, K_m = 2.7 mM), sucrose (4.6–37 mM, K_m = 20 mM), and isomaltose (0.46–3.7 mM, K_m = 4.5 mM). The results on Lineweaver–Burk plots revealed a fully competitive type of inhibition on each α-glucosidase.

Table 1 IC_{50} and K_i values of salacinol (**1**), kotalanol (**2**), acarbose, and voglibose for rat small intestinal disaccharidase.

	IC_{50} [K_i] (μg/ml)			
	Salacinol (**1**)	Kotalanol (**2**)	Acarbose	Voglibose
Maltase[a]	3.2 [0.31]	2.8 [0.23]	1.3 [0.12]	0.32 [0.032]
Sucrase[b]	0.84 [0.32]	0.58 [0.18]	1.1 [0.37]	0.059 [0.018]
Isomaltase[c]	0.59 [0.47]	1.9 [1.8]	100 [75]	0.56 [0.41]
Trehalase[d]	>400	>400	>400	190

Substrate: a) maltose (37 mM), b) sucrose (37 mM), c) isomaltose (3.7 mM), d) trehalose (3.7 mM).

Inhibitory effects of salacinol (1) on serum glucose levels in maltose- and sucrose-loaded rats

The effects of salacinol (**1**) on the increases in serum glucose levels in maltose- and sucrose-loaded rats were examined. As shown in Table 2, **1** showed stronger inhibition of the increase in serum glucose levels in maltose- and sucrose-loaded rats than acarbose. Therefore, we concluded that **1** and **2** are potent α-glucosidase inhibitors isolated from these natural medicines and are the constituents responsible for the antidiabetic effects of the Ayurvedic traditional medicines, *S. reticulata* and *S. oblonga*.

Table 2 Inhibitory effects of salacinol (**1**) and acarbose on serum glucose levels in maltose- or sucrose-loaded rats.

Treatment	Dose (mg/kg, p.o.)	*N*	Serum glucose (mg/dl)		
			0.5 h	1.0 h	2.0 h
Maltose-loaded					
Normal	—	5	56.8 ± 4.3**	60.1 ± 2.8**	65.4 ± 4.4*
Control	—	6	197.8 ± 8.8	134.5 ± 7.5	90.6 ± 4.8
Salacinol (**1**)	10	6	171.0 ± 5.3	129.6 ± 6.6	100.3 ± 5.3
	25	4	137.7 ± 7.6**	126.8 ± 6.1	101.4 ± 8.9
Acarbose	100	6	163.0 ± 6.0	132.4 ± 5.3	102.0 ± 5.2
	200	6	135.6 ± 12.6**	117.6 ± 6.4	103.8 ± 6.1
Sucrose-loaded					
Normal	—	5	66.0 ± 4.1**	74.0 ± 3.4**	78.4 ± 2.0**
Control	—	7	174.6 ± 5.1	149.7 ± 5.1	125.1 ± 4.6
Salacinol (**1**)	1.25	5	128.0 ± 10.0**	118.4 ± 4.9**	115.0 ± 3.8
	2.5	5	126.4 ± 3.2**	122.4 ± 2.0**	121.8 ± 2.7
	5.0	4	102.6 ± 3.8**	105.8 ± 3.9**	102.8 ± 2.9**
Normal	—	5	74.6 ± 3.4**	89.4 ± 4.4**	79.7 ± 6.6
Control	—	5	153.1 ± 4.6	143.5 ± 6.1	95.0 ± 3.3
Acarbose	5	5	126.1 ± 6.9*	137.2 ± 8.3	113.8 ± 7.2
	10	5	100.8 ± 5.2**	118.2 ± 2.9*	96.8 ± 3.1

Male Wistar rats weighing 130–170 g were fasted for 20–24 h but allowed water *ad libitum*. The test samples were suspended in 5 % acacia solution (5 ml/kg), and then orally administered to the rats. Thirty min later, a water solution (5 ml/kg) of sucrose (1.0 g/kg) or maltose (1.0 g/kg) was orally administered. Blood (ca. 0.4 ml) was collected from the infraorbital venous plexus under light ether anesthesia at 0.5, 1.0, and 2.0 h after administration of sugar, and the serum glucose concentration was determined by the glucose-oxidase method (Glucose CII-test Wako, Wako Pure Chemical Industries). Values represent the means ± SEM (*$p < 0.05$, **$p < 0.01$).

ALDOSE REDUCTASE INHIBITORS

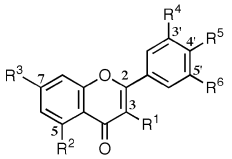
Aldose reductase as a key enzyme in the polyol pathway has been reported to catalyze the reduction of glucose to sorbitol. Sorbitol does not readily diffuse across cell membranes, and the intracellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as peripheral neuropathy, retinopathy, and cataracts.

The methanolic extracts from *Myrcia* (*M.*) *multiflora* (leaves), *Chrysanthemum indicum* (flowers), *Centella asiatica* (aerial parts), etc. showed potent inhibitory activities against rat lens aldose reductase [11–14]. As the leaves of *M. multiflora* have been extensively used as a specific remedy for diabetes, it goes by the popular name "plant insulin". However, no chemical and pharmacological studies of this plant have been reported as far as we know. By bioassay-guided separation of the ethyl acetate-soluble fraction using aldose reductase inhibitory activity, we have isolated new flavanone glucosides, myrciacitrins I–V, and new acetophenone glucosides, myrciaphenones A and B, together with five known flavonol glyco-

sides [11,12]. These flavonoid glycosides were found to strongly inhibit rat lens aldose reductase. Among them, three known compounds, quercitrin (**25**, IC_{50} = 0.15 μM) guaijaverin (**26**, 0.18 μM) and desmanthin-1 (**32**, 0.082 μM), showed the most potent activity. The activity of **32** was equivalent to that of a commercial synthetic aldose reductase inhibitor, epalrestat (0.072 μM) [11,12].

To clarify the structural requirements of flavonoids for rat lens aldose reductase inhibitory activity, various flavonoids were examined. As shown in Table 3, among the flave constituents, 3′,4′-dihydroxyflavone (**9**), 3′,4′,7-trihydroxyflavone (**10**), luteolin (**14**), and luteolin 7-*O*-β-D-glucopyranoside (**15**) potently inhibited the activity (IC_{50} = 0.30–0.99 μM), while flavone (**4**) and tectochrysin (**7**) lacked the activity (>100 μM). In addition, the flavones lacking the 5-hydroxyl group showed activities equivalent to those of flavones having the 5-hydroxyl group (ex. **5** ≒ **6**, **8** ≒ **11**). The activities of 7-*O*-glucosyl flavones were weaker than those of their aglycons (**12** < **11**, **15** < **14**, **17** < **14a**). Furthermore, the activities of the flavones having the catechol type moiety at the B ring (the 3′,4′-dihydroxyl groups) were stronger than those of corresponding monohydroxyl, mono- or dimethylated compounds (**10** > **8**, **14** > **11** and **14a**–**14d**, **15** > **17**).

Table 3 Inhibitory effects of flavones (**4**–**17**) and flavonols (**18**–**32**) on rat lens aldose reductase.



	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50} (μM)
Flavone (**4**)	H	H	H	H	H	H	>100 (16)[b]
7-Hydroxyflavone (**5**)	H	H	OH	H	H	H	10
Chrysin (**6**)	H	OH	OH	H	H	H	8.5
Tectochrysin (**7**)	H	OH	OCH_3	H	H	H	>100 (34)[b]
4′,7-Dihydroxyflavone (**8**)	H	H	OH	H	OH	H	3.8
3′,4′-Dihydroxyflavone (9)	**H**	**H**	**H**	**OH**	**OH**	**H**	**0.37**
3′,4′,7-Trihydroxyflavone (10)	**H**	**H**	**OH**	**OH**	**OH**	**H**	**0.30**
Apigenin (**11**)	H	OH	OH	H	OH	H	2.2
11a	H	OH	OCH_3	H	OCH_3	H	>30 (39)[a]
Apigenin 7-*O*-Glc (**12**)	H	OH	O-Glc	H	OH	H	23
Acacetin 7-*O*-Rut (**13**)	H	OH	O-Glc(6 → 1)Rha	H	OCH_3	H	4.7
Luteolin (14)	**H**	**OH**	**OH**	**OH**	**OH**	**H**	**0.45**
Diosmetin (**14a**)	H	OH	OH	OH	OCH_3	H	8.5
Pilloin (**14b**)	H	OH	OCH_3	OH	OCH_3	H	12
14c	H	OH	OCH_3	OCH_3	OCH_3	H	72
14d	H	OCH_3	OCH_3	OCH_3	OCH_3	H	>30 (30)[a]
Luteolin 7-*O*-Glc (15)	**H**	**OH**	**O-Glc**	**OH**	**OH**	**H**	**0.99**
Luteolin 7-*O*-GlcA (**16**)	H	OH	O-GlcA	OH	OH	H	3.1
Diosmetin 7-*O*-Glc (**17**)	H	OH	O-Glc	OH	OCH_3	H	23
3-Hydroxyflavone (**18**)	OH	H	H	H	H	H	>30 (14)[a]
Izalpinin (**19**)	OH	OH	OCH_3	H	H	H	>100 (38)[b]
Kaempferol (**20**)	OH	OH	OH	H	OH	H	10
Kaempferol 3-*O*-GlcA (**21**)	O-GlcA	OH	OH	H	OH	H	5.1
Quercetin (**22**)	OH	OH	OH	OH	OH	H	2.2
Rhamnetin (**22a**)	OH	OH	OCH_3	OH	OH	H	2.7
Tamarixetin(**22b**)	OH	OH	OH	OH	OCH_3	H	11
22c	**OCH_3**	**OH**	**OCH_3**	**OH**	**OH**	**H**	**0.82**
Ombuine (**22d**)	OH	OH	OCH_3	OH	OCH_3	H	6.0

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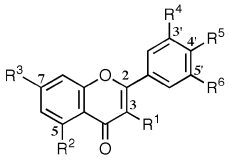
Table 3 (*Continued*).



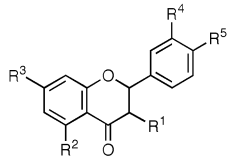
	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50} (μM)
Ayanin (**22e**)	OCH_3	OH	OCH_3	OH	OCH_3	H	34
22f	OH	OH	OCH_3	OCH_3	OCH_3	H	73
22g	OCH_3	OH	OCH_3	OCH_3	OCH_3	H	25
22h	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	H	>100 (24)[b]
Isoquercitrin (**23**)	O-Glc	OH	OH	OH	OH	H	4.5
Hyperin (**24**)	O-Gal	OH	OH	OH	OH	H	3.0
Quercitrin (25)	**O-Rha**	**OH**	**OH**	**OH**	**OH**	**H**	**0.15**
Guaijaverin (26)	**O-Ara**	**OH**	**OH**	**OH**	**OH**	**H**	**0.18**
Quercitrin 3,7-di-*O*-Glc (**27**)	O-Glc	OH	O-Glc	OH	OH	H	84
Rutin (**28**)	O-Glc(6 → 1)Rha	OH	OH	OH	OH	H	9.0
Rhamnetin 3-*O*-Rut (**28a**)	O-Glc(6 → 1)Rha	OH	OCH_3	OH	OH	H	21
Ombuine 3-*O*-Rut (**28b**)	O-Glc(6 → 1)Rha	OH	OCH_3	OH	OCH_3	H	41
28c	O-Glc(6 → 1)Rha	OH	OCH_3	OCH_3	OCH_3	H	88
Fisetin (**29**)	OH	H	OH	OH	OH	H	3.7
Myricetin (**30**)	OH	OH	OH	OH	OH	OH	29
Mearnsetin (**30a**)	OH	OH	OH	OH	OCH_3	OH	19
30b	OH	OH	OCH_3	OH	OH	OH	21
30c	OCH_3	OH	OCH_3	OH	OH	OH	12
30d	OH	OH	OCH_3	OH	OCH_3	OH	24
30e	OH	OH	OCH_3	OCH_3	OCH_3	OH	44
30f	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	>100 (12)[b]
Myricitrin (**31**)	O-Rha	OH	OH	OH	OH	OH	3.8
Mearncitrin (**31a**)	O-Rha	OH	OH	OH	OCH_3	OH	3.8
31b	O-Rha	OH	OCH_3	OH	OCH_3	OH	48
31c	O-Rha	OH	OH	OCH_3	OCH_3	OH	21
31d	O-Rha	OH	OCH_3	OCH_3	OCH_3	OH	71
31e	O-Rha	OH	OCH_3	OCH_3	OCH_3	OCH_3	71
Desmanthin-1 (32)	**O-(2′-galloyl)-Rha**	**OH**	**OH**	**OH**	**OH**	**OH**	**0.082**

Glc: β-D-glucopyranosyl; Gal: β-D-galactopyranosyl; Rha: α-L-rhamnopyranosyl; Ara: α-L-arabinopyranosyl; Rut: Glc(6 → 1)Rha. Values in parentheses represent the inhibition (%) at [a]30 μM and [b]100 μM.

Among the flavonols, **22c**, (**25**), (**26**), and (**32**) were found to show potent inhibition (0.082–0.82 μM), and these results supported the above suggestion. In addition, the flavonols (having the 3-hydroxyl group) and flavanones (single bond at the 2–3 position) showed weak activities as compared with their corresponding flavones (**11 > 20**, **14 > 22** and **36**) (Table 4). These compounds having the catechol type moiety at the B ring showed stronger activities than those of the pyrogallol type moiety (**22 > 30**, **22a > 30b**, **22c > 30c**, **25 > 31**), except for **32**. However, isoflavones [daidzein (24 μM), daidzin (>100 μM), genistein (20 μM), genistin (37 μM), etc.] and flavan-3-ols [(+)-catechin, (–)-epicatechin, (–)-epigallocatechin] (>30 μM) showed weaker activity than the flavones, flavonols, and flavanones [15].

These results indicated the following structural requirements of flavonoids for aldose reductase inhibitory activity: (1) the 5-hydroxyl moiety has no effect; (2) the 3-hydroxyl and 7-*O*-glucosyl moieties reduce the activity; (3) the 2–3 double bond enhances the activity; and (4) the flavones and

Table 4 Inhibitory effects of flavanones (**33**–**38**) and a flavanonol (**39**) on rat lens aldose reductase.



	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)
Flavanone (**33**)	H	H	H	H	H	>100 (38)[b]
Liquiritigenin (**34**)	H	H	OH	H	OH	3.4
34a	H	H	OCH_3	H	OH	1.9
Liquiritin (**35**)	H	H	OH	H	O-Glc	9.5
35a	H	H	OCH_3	H	O-Glc	30
Eriodictyol (**36**)	H	OH	OH	OH	OH	7.7
(2*S*)-Eriodictyol 7-*O*-GlcA (**37**)	H	OH	O-GlcA	OH	OH	2.1
(2*R*)-Eriodictyol 7-*O*-GlcA (**38**)	H	OH	O-GlcA	OH	OH	1.5
Fustin (**39**)	OH	H	OH	OH	OH	14

flavonols having the catechol type moiety at the B ring (the 3′,4′-dihydroxyl groups) exhibit stronger activities than those of pyrogallol-type moiety (the 3′,4′,5′-trihydroxyl groups).

In conclusion, we have isolated salacinol (**1**) and kotalanol (**2**) with unique thiosugar sulfonium sulfate inner salt structures from the antidiabetic Ayurvedic traditional medicines, *S. reticulata* and *S. oblonga*, while various flavonoids with potent inhibitory activity against rat lens aldose reductase such as quercitrin (**25**), guaijaverin (**26**), and desmantin-1 (**32**) were isolated from *M. multiflora* and various medicinal herbs. Salacinol (**1**) and kotalanol (**2**) showed potent inhibitory activities against intestinal α-glucosidase, and the inhibitory effects of **1** on the increase in serum glucose levels in maltose and sucrose-loaded rats were found to be more potent than those of acarbose. In addition, some structural requirements of flavonoids for aldose reductase inhibitory activity were clarified.

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