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Absolute Stereostructure of Potent α-Glucosidase Inhibitor, Salacinol, with Unique Thiosugar Sulfonium Sulfate Inner Salt Structure from *Salacia reticulata*

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Abstract—A most potent α-glucosidase inhibitor named salacinol has been isolated from an antidiabetic Ayurvedic traditional medicine, *Salacia reticulata* WIGHT, through bioassay-guided separation. The absolute stereostructure of salacinol was determined on the basis of chemical and physicochemical evidence, which included the alkaline degradation of salacinol to 1-deoxy-4-thio-D-arabinofuranose and the X-ray crystallographic analysis, to be the unique spiro-like configuration of the inner salt comprised of 1-deoxy-4-thio-D-arabinofuranosyl sulfonium cation and 1′-deoxy-D-erythrosyl-3′-sulfate anion. Salacinol showed potent inhibitory activities on several α-glucosidases, such as maltase, sucrase, and isomaltase, and the inhibitory effects on serum glucose levels in maltose- and sucrose-loaded rats (in vivo) were found to be more potent than that of acarbose, a commercial α-glucosidase inhibitor.

Introduction

The Hippocrateaceae plant[1] *Salacia reticulata* WIGHT ('Kotala himbutu' in Singhalese), a large woody climbing plant in the submontane forests, is distributed in Sri Lanka and the south region of India. The roots and stems of *S. reticulata* have been extensively used for the treatments of rheumatism, gonorrhea, and skin diseases, particularly as a specific remedy at the initial stages of diabetes in the Ayurvedic system of Indian traditional medicine.[2] The aqueous extract from the root bark of Sri Lankan *S. reticulata* was reported to show hypoglycemic activity in rats,[3] while the aqueous extract was also reported to produce a significant lowering of plasma glucose in streptozotocin-induced diabetic rats.[4] As the constituents of this plant, many triterpenes as well as hydrocarbon, sitosterol, mangiferin, and gutta-percha were isolated from the root and stem barks.[5] However, pharmacologically active components were left uncharacterized.

In the course of our studies on antidiabetogenic compounds from natural medicines and medicinal foodstuffs,[6] we found that the aqueous methanolic extract of Sri Lankan *S. reticulata* did not show hypoglycemic effects on oral D-glucose-loaded rats and alloxan-induced diabetic mice, while the extract and the water-soluble portion showed potent hypoglycemic effect in oral sucrose-loaded rats and α-glucosidase inhibitory effect. Through bioassay-guided separation using α-glucosidase inhibitory activities, we isolated potent α-glucosidase inhibitors termed salacinol (**1**)[7] and kotalanol[8] from the water-soluble portion together with several phenolic compounds.[9] Furthermore, **1** and kotalanol were also isolated from Indian *S. oblonga* and Thai *S. chinensis* together with several new triterpenes with aldose reductase inhibitory activity.[10]

This paper presents a full account of the isolation and structural elucidation of salacinol (**1**) from the roots and stems of Sri Lankan *S. reticulata*.[11] In addition, we describe the inhibitory properties of the extracts and **1** on the increase of serum glucose levels in several sugar-loaded rats (in vivo) and the inhibitory activities of **1** on various glucosidase (in vitro).

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Table 1. Inhibitory effects of the MeOH extract from *Salacia reticulata* on serum glucose levels in several sugar-loaded rats

Treatment	Dose (mg/kg, po)	*N*	Serum glucose (mg/dL)		
			0.5 h	1.0 h	2.0 h
Maltose-loaded					
Normal	—	12	81.3±4.2**	97.2±4.3**	94.5±4.1**
Control	—	12	212.1±6.2	183.5±8.6	129.3±2.6
MeOH ext.	50	6	160.0±11.9*	164.7±4.8	142.9±4.5
	100	6	168.8±9.2**	160.9±7.0	141.3±3.3
	200	6	116.9±6.9**	132.4±5.7**	130.3±3.1
Sucrose-loaded					
Normal	—	12	77.2±3.3**	94.8±5.0**	92.7±4.7**
Control	—	12	182.9±5.5	153.0±3.9	129.0±2.8
MeOH ext.	50	6	142.3±7.0**	128.4±2.7**	123.6±1.9
	100	6	123.4±9.7**	118.6±6.8**	125.2±3.7
	200	6	97.2±4.0**	105.3±4.4**	104.2±3.2**
Glucose-loaded					
Normal	—	12	85.9±4.0**	88.9±2.9**	93.3±3.2
Control	—	12	191.2±5.5	156.1±4.5	120.6±2.2
MeOH ext.	50	6	169.6±9.9	163.7±12.2	146.4±19.1
	100	6	197.8±9.4	179.2±11.9	148.4±18.8
	200	6	198.0±13.7	200.7±21.5**	167.1±33.9

Each value represents the mean±SEM. Significantly different from the control: $*p<0.05$, $**p<0.01$.

Results and Discussion

The isolation of the chemical constituents from *S. reticulata* was carried out through the following procedure. The dried roots and stems of *S. reticulata* (collected in Sri Lanka) were extracted with methanol under reflux. The methanolic extract was found to inhibit the increase of serum glucose levels in maltose- and sucrose-loaded rats as shown in Table 1, whereas the methanolic extract did not show the inhibitory effects on serum glucose levels in glucose-loaded rats. In addition, the effect of the methanolic extract on serum glucose levels in alloxan-induced diabetic mice, which is known to be a experimental model for insulin-dependent diabetics, was examined. However, the methanolic extract lacked hypoglycemic effect at the single administration of 3000 mg/kg (po) as shown in Table 2. The methanolic extract was then partitioned into an ethyl acetate (AcOEt) and water (H_2O) mixture to give an AcOEt-soluble fraction and H_2O-soluble fraction. The H_2O-soluble fraction was found to show inhibitory effect on the increase of serum glucose levels in sucrose-loaded rats, but the AcOEt-soluble fraction did not affect at a dose of 50 mg/kg (Table 3).

The results of above-mentioned in vivo screening test led us to confirm that the mechanism of antidiabetic effects of this natural medicine was suggested to be inhibitory activity for small intestinal α-glucosidase, and the α-glucosidase inhibitory constituent was concentrated in the H_2O-soluble fraction (maltase: MeOH extract $IC_{50}=42$ μg/mL, H_2O-soluble fraction $IC_{50}=35$ μg/mL, sucrase: MeOH extract $IC_{50}=32$ μg/mL, H_2O-soluble fraction $IC_{50}=26$ μg/mL).

The isolation of the α-glucosidase inhibitor from the H_2O-soluble fraction was carried out by bioassay-guided separation using inhibition indices for maltase and sucrase from small intestinal brush border membrane

Table 2. Effects of the MeOH extract from *Salacia reticulata* on serum glucose levels in alloxan-induced diabetic mice

Treatment	Dose (mg/kg)	*N*	Serum glucose (mg/dL)		
			0 h	1 h	3 h
Control	—	8	689.3±29.2	604.3±15.4	580.4±10.0
MeOH ext.	250 (po)	8	684.8±26.7	565.6±20.2	616.4±18.4
Insulin	1 (U/kg, ip)	8	690.3±40.3	107.1±19.0**	107.4±8.9**
Control	—	8	625.3±19.3	527.4±31.4	533.6±18.0
MeOH ext.	1500 (po)	8	618.6±27.0	550.1±20.7	527.3±22.4
	3000 (po)	8	610.1±25.3	547.9±43.0	583.1±36.9

Each value represents the mean±SEM. Significantly different from the control: $**p<0.01$.

Table 3. Inhibitory effects of AcOEt- and H_2O-soluble fractions from *Salacia reticulata* on serum glucose levels in sucrose-loaded rats

Treatment	Dose (mg/kg, po)	*N*	Serum glucose (mg/dL)
			0.5 h
Normal	—	5	82.7±5.9**
Control	—	5	168.4±9.3
AcOEt-soluble fraction	50	5	162.0±3.7
	200	5	140.6±5.8*
H_2O-soluble fraction	10	6	154.4±7.0
	25	6	128.5±2.0**
	50	5	117.4±5.4**
	100	6	106.1±4.6**

Each value represents the mean±SEM. Significantly different from the control: $*p<0.05$, $**p<0.01$.

vesicles (Chart 1). The H_2O-soluble fraction was suspended with methanol and then filtered through Kiriyama funnel to give a methanol-soluble phase and a residue. The inhibitory activities were concentrated in the methanol-soluble phase (maltase: $IC_{50}=30$ μg/mL, sucrase: $IC_{50}=18$ μg/mL), so that the methanol-soluble phase was subjected to ordinary-phase silica gel chromatography ($CHCl_3$–MeOH–H_2O) to give eight fractions (Fr. 1–8). The active fractions [Fr. 3 and 4 (maltase: $IC_{50}=60$, 41 μg/mL, sucrase: $IC_{50}=15$, 6.7 μg/mL)] and Fr. 2 were successively subjected to ODS and NH column chromatography and finally HPLC [Shodex SC1011 (Ca^{2+}), SP0811 (Pb^{2+}), and YMC-Pack Polyamine II] to furnish salacinol (**1**, 0.025% from natural medicine), dulcitol (0.047%), D-glucose (0.100%), D-fructose (0.138%), sucrose (0.099%), and glycerol (0.010%).

Absolute stereostructure of salacinol (1). Salacinol (**1**) was isolated as colorless prisms of mp 187–189 °C (EtOH) with positive optical rotation ($[\alpha]_D^{28}$ +4.9°) and was deduced to possess a sulfate group by the positive potassium rhodizonate test.[12] The positive-ion fast atom bombardment (FAB)-MS of **1** showed a quasimolecular ion peaks at m/z 357 $(M+Na)^+$ and 335 $(M+H)^+$ in addition to a fragment ion peak at m/z 255 $(M-SO_3+H)^+$, while a quisimolecular ion peak was observed at m/z 333 $(M-H)^-$ in the negative-ion FAB-MS. The molecular formula of **1** has been shown to be $C_9H_{18}O_9S_2$ by high-resolution MS analysis. The IR spectrum of **1** showed absorption bands due to hydroxyl (3417, 1073, 1019 cm^{-1}) and sulfate (1262, 1238, 801 cm^{-1}) functions. The ^1H and ^{13}C NMR (Table 4) spectra of **1** showed signals assignable to four methylenes linking to a heteroatom {δ (pyridine-d_5): 4.33 (2H, br s,

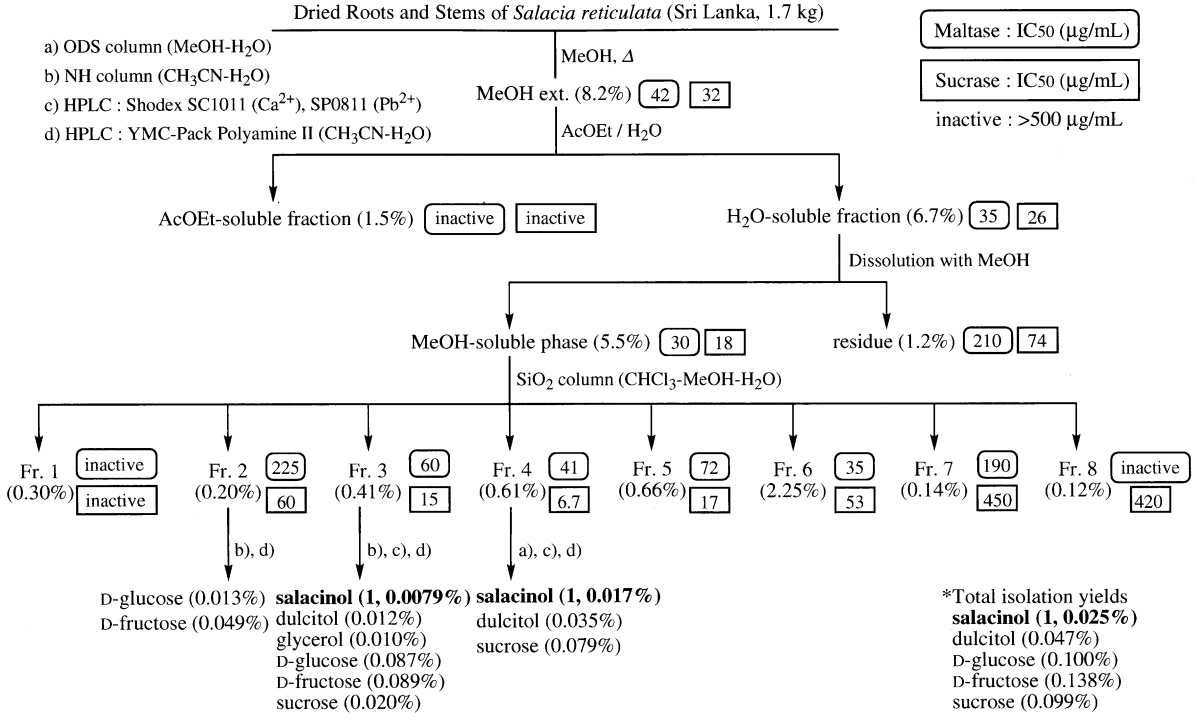

Chart 1. Bioassay-guided separation from the died roots and stems of S*alacia reticulata* using α-glucosidase inhibitory activities.

Table 4. ^1H NMR and ^{13}C NMR data for salacinol (**1**)

Solvent	Pyridine-d_5		CD_3OD		CD_3OH
	^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR	^{13}C NMR
C-1	4.33 (2H, br s)	50.7	3.83 (2H, br s)	51.44	51.45
C-2	5.10 (1H, br s)	78.5	4.60 (1H, br dd, J=ca. 3, 5 Hz)	79.24	79.36
C-3	5.12 (1H, dd-like)	79.0	4.40 (1H, dd, J=1.2, 2.7 Hz)	79.75	79.90
C-4	4.69 (1H, t-like)	72.6	4.01 (1H, br dd, J=ca. 5, 7 Hz)	73.43	73.50
C-5	4.51 (1H, dd, J=8.0, 11.6 Hz)	60.2	3.95 (1H, dd, J=7.1, 10.5 Hz)	60.95	61.06
	4.54 (1H, dd, J=6.8, 11.6 Hz)		4.03 (1H, dd, J=5.2, 10.5 Hz)		
C-1′	4.62 (1H, dd, J=4.2, 13.1 Hz)	52.6	3.86 (1H, dd, J=6.4, 13.1 Hz)	52.35	52.38
	4.76 (1H, dd, J=4.9, 13.1 Hz)		3.97 (1H, dd, J=3.6, 13.1 Hz)		
C-2′	4.99 (1H, ddd, J=4.2, 4.9, 7.6 Hz)	67.6	4.34 (1H, ddd, J=3.6, 6.4, 7.6 Hz)	67.69	67.81
C-3′	5.25 (1H, ddd, J=3.7, 3.9, 7.6 Hz)	79.4	4.29 (1H, ddd, J=3.4, 3.5, 7.6 Hz)	80.56	80.59
C-4′	4.37 (1H, dd, J=3.9, 11.6 Hz)	62.2	3.83 (1H, dd, J=3.4, 12.2 Hz)	61.75	61.88
	4.60 (1H, dd, J=3.7, 11.6 Hz)		3.94 (1H, dd, J=3.5, 12.2 Hz)		

^1H NMR measured at 500 MHz. ^{13}C NMR measured at 125 MHz.

1-H_2), [4.37 (dd, J = 3.9, 11.6 Hz), 4.60 (dd, J = 3.7, 11.6 Hz), 4′-H_2], [4.51 (dd, J = 8.0, 11.6 Hz), 4.54 (dd, J = 6.8, 11.6 Hz), 5-H_2], [4.62 (dd, J = 4.2, 13.1 Hz), 4.76 (dd, J = 4.9, 13.1 Hz), 1′-H_2]} and five methines bonding to a heteroatom [δ (pyridine-d_5): 4.69 (t-like, 4-H), 4.99 (ddd, J = 4.2, 4.9, 7.6 Hz, 2′-H), 5.10 (br s, 2-H), 5.12 (dd-like, 3-H), 5.25 (ddd, J = 3.7, 3.9, 7.6 Hz, 3′-H)]. The proton and carbon signals in the ^{1}H and ^{13}C NMR spectra of **1** were completely assigned with the aid of disortionless enhancement by polarization transfer (DEPT), homo- and hetero-correlation spectroscopy (^{1}H–^{1}H and ^{13}C–^{1}H COSY), heteronuclear multiple bond correlation (HMBC), and homo- and hetero-nuclear Hartmann–Hahn spectroscopy (^{1}H–^{1}H and ^{13}C–^{1}H HOHAHA) experiments and the planar structure of **1** was elucidated. Thus, the H–H COSY experiment on **1** indicated the presence of two partial structures (1-C–5-C and 1′-C–4′-C) written in bold line as shown in Figure 1. In the HMBC experiment, long-range correlations were observed between the 1′-proton and the 4-carbon, between the 1-proton and 1′-carbon, and between the 1′-proton and 1-carbon, respectively (Fig. 1).

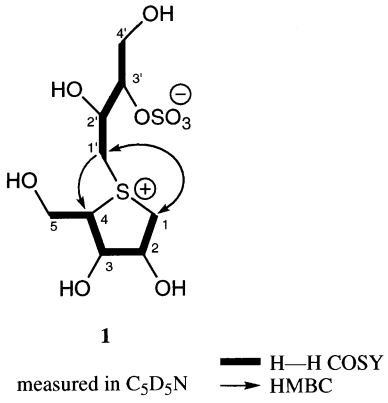

Figure 1. H–H COSY and HMBC correlations of salacinol (**1**).

In order to facilitate the locations of free hydroxyl groups in **1**, we compared the ^{13}C NMR spectra taken in CD_3OH and CD_3OD. The deuterium shifts were observed at five carbon signals due to the 2, 3, 5, 2′, and 4′ positions by 0.11–0.15 ppm, while the signal due to the sulfate-bearing carbon (3′-C) was slightly changed as shown in Figure 2.

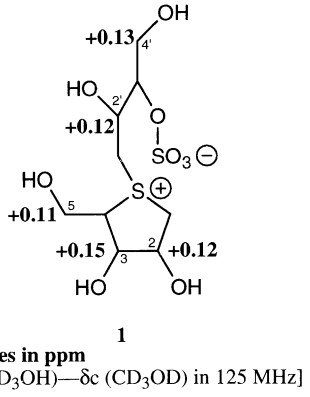

Figure 2. Aplication of deuterium shift rule for salacinol (**1**).

Next, the relative stereostrucuture of **1** was clarified by the nuclear Overhauser enhancement spectroscopy (NOESY) experiment and X-ray crystallographic analysis. Namely, nuclear Overhauser effect (NOE) was observed between the 2-proton and the 4-proton. As shown in X-ray molecular structure (computer-generated perspective drawing), the molecular conformation of **1** was found to show the unique spiro-like configuration of the inner salt comprised of 1-deoxy-4-thioarabinofuranosyl sulfonium cation and 1′-deoxyerythrosyl-3′-sulfate anion (Fig. 3). Finally, alkaline treatment of **1** with 1% sodium methoxide (NaOMe)-methanol liberated 1-deoxy-4-thio-D-arabinofuranose (**1a**), which was identified with an authentic sample prepared from D-xylose using the previously reported method.[13] Consequently, the absolute stereostructure of salacinol (**1**) was determined as shown.

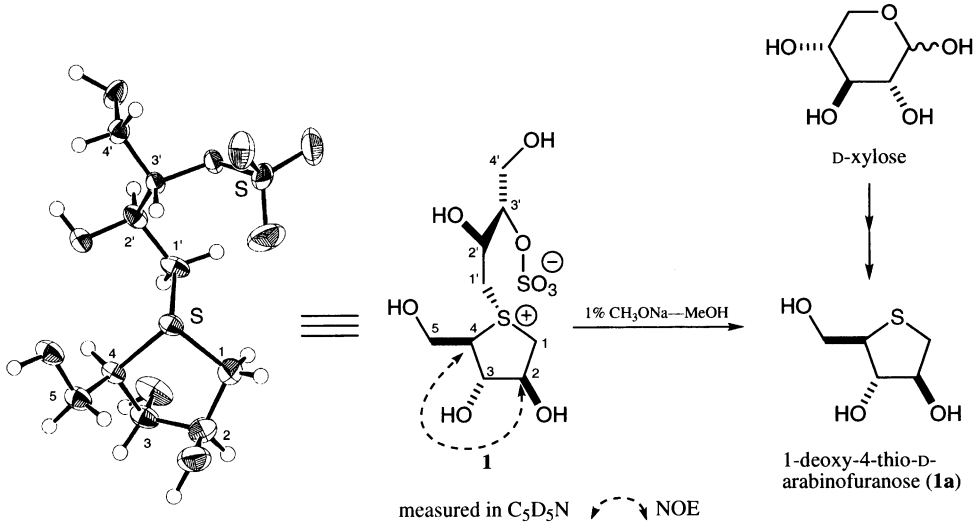

Figure 3. Absolute streostructure and conformation of salacinol (**1**).

α-Glucosidase inhibitory activities of salacinol (1) and related compounds (1b, 1c). Salacinol (**1**) showed the competitive inhibition for the small intestinal α-glucosidase in vitro and other constituents such as dulcitol did not exhibit the activity. Table 5 indicated 50% inhibitory concentration (IC_{50}) values of **1** and commercial medicines as α-glucosidase inhibitors, acarbose and voglibose. The maltase inhibitory activity was slightly weaker than those of acarbose and voglibose (**1**: $IC_{50} = 3.2$ μg/mL; acarbose: 1.3 μg/mL; voglibose: 0.32 μg/mL) and the sucrase inhibitory activity was similar to that of acarbose (**1**: 0.84 μg/mL; acarbose: 1.1 μg/mL), while the isomaltase inhibitory activity was more potent than that of acarbose and equivalent to that of voglibose (**1**: 0.59 μg/mL; acarbose: 100 μg/mL; voglibose: 0.56 μg/mL).

On the other hand, 1-deoxy-4-thio-D-arabinofuranose (**1a**) showed no activity (IC_{50}: maltase >400 μg/mL, sucrase >400 μg/mL). This evidence suggested that the side chain having sulfate anion was essential for the activity.

In order to examine the types of inhibitions of maltase, sucrase, and isomaltase by salacinol (**1**), the small intestinal brush border membrane vesicles were incubated with increasing concentrations of maltose (3.1–37 mM), sucrose (4.6–37 mM), and isomaltose (0.46-3.7 mM). The results plotted according to Lineweaver–Burk revealed a fully competitive type of inhibition on each α-glucosidase and K_i values of **1** on maltase, sucrace, and isomaltase were 0.31, 0.32, and 0.47 μg/mL, respectively (Fig. 4 and Table 6).

Inhibitory effects of salacinol (1) on serum glucose levels in maltose- and sucrose-loaded rats. The effects of salacinol (**1**) on increase of serum glucose levels in maltose- and sucrose-loaded rats were examined. As shown in Table 7, **1** showed stronger inhibition of the increase of serum glucose levels in maltose- and sucrose-loaded rats

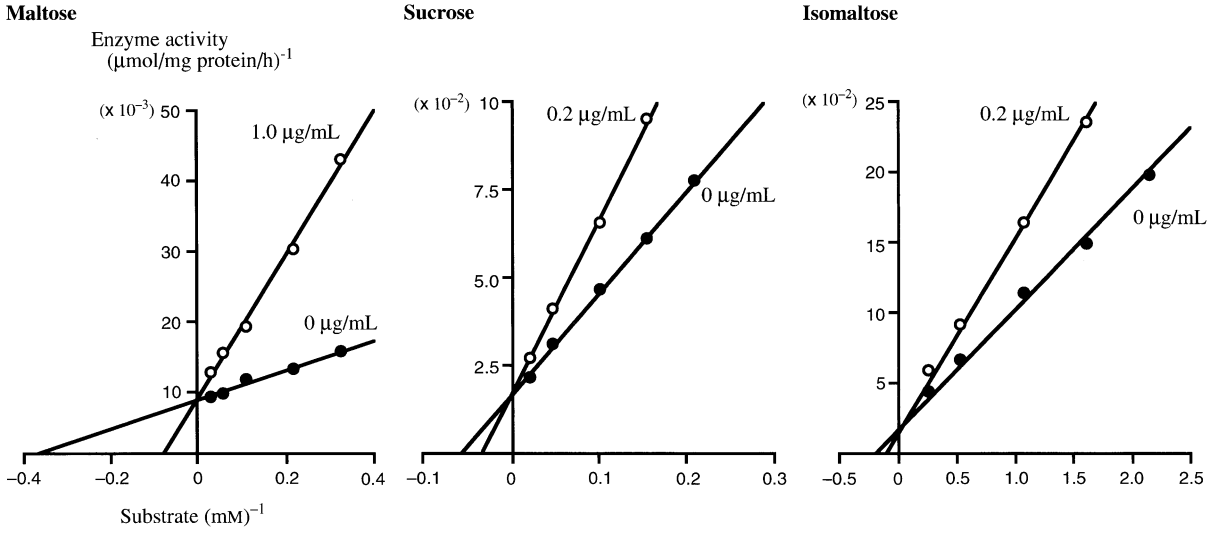

Figure 4. Lineweaver–Burk plots of the inhibition of rat intestinal maltase, sucrase, and isomaltase activities by salacinol (**1**).

Table 5. IC_{50} values of salacinol (**1**), **1a**, acarbose, and voglibose for rat small intestinal disaccharidase and β-glucosidase

Enzyme (Substrate concentration)	IC_{50} (μg/mL)			
	Salacinol (**1**)	**1a**	Acarbose	Voglibose
Maltase (37 mM)	3.2	>400	1.3	0.32
Sucrase (37 mM)	0.84	>400	1.1	0.059
Isomaltase (3.7 mM)	0.59	—	100	0.56
Trehalase (3.7 mM)	>400	—	>400	190
β-Glucosidase (2.5 mM)[a]	>400	—	>400	>400

[a] *p*-Nitrophenyl-β-D-glucopyranoside.

Table 6. K_i values of salacinol (**1**), acarbose, and voglibose for rat small intestinal disaccharidase

Substrate	K_m (M)	K_i (μg/mL)		
		Salacinol (**1**)	Acarbose	Voglibose
Maltose	2.7×10^{-3}	0.31	0.12	0.032
Sucrose	2.0×10^{-2}	0.32	0.37	0.018
Isomaltose	4.5×10^{-3}	0.47	75	0.41

Table 7. Inhibitory effects of salacinol (**1**) and acarbose on serum glucose levels in maltose- and sucrose-loaded rats

Treatment	Dose (mg/kg, po)	*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**)	5	6	175.5±12.4	136.0±5.4	109.1±6.1
	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	25	6	175.6±9.0	138.7±8.1	100.3±5.6
	50	6	173.9±8.8	137.9±3.8	104.8±4.3
	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	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	1	5	150.9±9.4	146.6±4.7	93.4±3.6
	2	5	141.2±6.2	152.5±7.1	106.3±3.6
	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

Each value represents the mean±SEM. Significantly different from the control: $*p<0.05$, $**p<0.01$.

than acarbose. Therefore, it is concluded that **1** is a potent α-glucosidase inhibitor isolated from this natural medicine and is a responsible constituent of the antidiabetic Ayurvedic traditional medicine 'Kotala himbutu', the roots and stems of *S. reticulata*.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter ($l=5$ cm); IR spectra, Shimadzu FTIR-8100 spectrometer; MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; X-ray crystallographic analysis, Rigaku AFC5R diffractionspectrometer; ^{1}H NMR spectra, JNM-LA500 (500 MHz) spectrometer; ^{13}C NMR spectra, JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index detector.

The following experimental conditions were used for chromatography: ordinary-phase 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); NH column chromatography, Chromatorex NH-DM1020 (Fuji Silysia Chemical, Ltd., 100–200 mesh). Packed column for HPLC: Shodex SC1011 (Ca^{2+}, 8.0×300 mm, i.d.), Shodex SP0810 (Pb^{2+}, 8.0×300 mm, i.d.), and YMC-Pack Polyamine II (4.6×250 mm, i.d. and 10×250 mm, i.d.). TLC, pre-coated 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); HPTLC, pre-coated TLC plates with Silica gel RP-18 WF_{254s} (Merck, 0.25 mm) (reversed-phase). Detection was done by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Extraction and isolation of salacinol (1) and known compounds from the dried stems of *Salacia reticulata*

The dried roots and stems of *S. reticulata* (1.7 kg, collected in Sri Lanka) was crushed and extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the MeOH extract (139 g, 8.2%), and a part of it (130 g) was partitioned in an AcOEt–H_2O (1:1) mixture. Removal of the solvent under reduced pressure gave the AcOEt-soluble fraction (23 g, 1.5%) and H_2O-soluble fraction (107 g, 6.7%). The H_2O-soluble fraction (50 g) was suspended with MeOH and then filtered through Kiriyama funnel to give a methanol-soluble phase (41 g, 5.5%) and residue (9 g, 1.2%). The methanol-soluble phase (31 g) was subjected to ordinary-phase silica gel chromatography [1.5 kg, $CHCl_3$-MeOH-H_2O (6:4:1→5:5:1→3:7:1) →MeOH] to give eight fractions [Fr. 1 (1.78 g), Fr. 2 (1.22 g), Fr. 3 (2.46 g), Fr. 4 (3.69 g), Fr. 5 (3.96 g), Fr. 6 (13.50 g), Fr. 7 (0.87 g), Fr. 8 (0.74 g)]. Fraction 2 (1.00 g) was subjected to NH column chromatography [30 g, CH_3CN–H_2O (70: 30, v/v) → H_2O] to give 70% aqueous CH_3CN-eluted fraction (Fr. 2-1, 400 mg), and H_2O-eluted fraction (Fr. 2-2, 600 mg). Fraction 2-1 (400 mg)

was further purified by HPLC [YMC-Pack Polyamine II, CH_3CN–H_2O (70:30, v/v)] to furnish D-glucose (25 mg, 0.013%) and D-fructose (96 mg, 0.049%). Fraction 3 (700 mg) was subjected to NH column chromatography [20 g, CH_3CN–H_2O (70: 30, v/v) → H_2O] to give 70% aqueous CH_3CN-eluted fraction (Fr. 3-1, 460 mg), and H_2O-eluted fraction (Fr. 3-2, 240 mg). Fraction 3-1 (460 mg) was further purified by HPLC [Shodex SC1011 (Ca^{2+}), Shodex SP0811 (Pb^{2+}), H_2O, column temperature 80 °C] and [YMC-Pack Polyamine II, CH_3CN–H_2O (70:30, v/v)] to furnish salacinol (**1**, 13 mg, 0.0079%), dulcitol (20 mg, 0.012%), glycerol (17 mg, 0.010%), D-glucose (152 mg, 0.087%), D-fructose (153 mg, 0.089%), and sucrose (34 mg, 0.020%). Fraction 4 (2.60 g) was subjected to reversed-phase silica gel column chromatography [50 g, MeOH→H_2O] to give MeOH-eluted fraction (Fr. 4-1, 1.50 g), and H_2O-eluted fraction (Fr. 4-2, 1.10 g). Fraction 4-2 (1.10 g) was further purified by HPLC [Shodex SC1011 (Ca^{2+}), Shodex SP0811 (Pb^{2+}), H_2O, column temperature 80 °C] and [YMC-Pack Polyamine II, CH_3CN–H_2O (70:30, v/v)] to furnish salacinol (**1**, 73 mg, 0.017%), dulcitol (149 mg, 0.035%), and sucrose (337 mg, 0.079%). Their known constituents were identified by comparison of their physical data ($[\alpha]_D$, IR, ^1H NMR, ^{13}C NMR) with those of commercially obtained samples.

Salacinol (**1**): Potassium rhodizonate test positive, colorless prisms from EtOH, mp 187–189 °C, $[\alpha]_D^{28}$ +4.9° (*c* 0.35, MeOH). High-resolution positive-ion FAB-MS: calcd for $C_9H_{18}O_9S_2Na$ $(M+Na)^+$: 357.0301. Found: 357.0290. IR (KBr): 3417, 1262, 1238, 1073, 1019, 801 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5 and CD_3OD) δ given in Table 4. ^{13}C NMR (125 MHz, pyridine-d_5, CD_3OD, and CD_3OH) δc given in Table 4. Positive-ion FAB-MS: *m*/*z* 357 $(M+Na)^+$, 335 $(M+H)^+$, 255 $(M-SO_3+H)^+$. Negative-ion FAB-MS: *m*/*z* 333 $(M-H)^-$.

X-ray crystallographic analysis on salacinol (1). X-ray crystal data of salacinol (**1**) were collected by a Rigaku AFC5R diffractmeter. The structure was solved by the direct method and refined with a full-matrix least-squares method.

Crystal data for salacinol (1). $C_9H_{18}O_9S_2$, $M=334.36$, monoclinic, space group $P2_1$, $a=6.433(3)$, $b=12.927(2)$, $c=8.372(3)$ Å, $\beta=93.68(3)°$, $V=694.8(4)$ Å^3, $Z=2$, μ(Mo-K$_\alpha$) = 4.05 cm^{-1}, $F(000)=352$, $D_c=1.598$ g/cm^{-3}, crystal dimensions: 0.15×0.20×0.20 mm. A total of 1812 reflections (1675 unique) were collected using the ω-2θ scan technique to a maximum 2θ value of 55°, and 1454 reflections with $I > 3\sigma(I)$ were used in the structure determination. Final R and R_w values were 0.034 and 0.038, respectively. The maximum and minimum peaks in the difference map were 0.37 and −0.24 e Å^{-3}, respectively. The data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 174565.

Alkaline hydrolysis of salacinol (1). A solution of salacinol (**1**, 15 mg, 45 μmol) in 1% NaOMe–MeOH (2.0 mL) was stirred under reflux for 3 h. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure gave a residue, which was purified by silica gel column chromatography [1.6 g, ($CHCl_3$–MeOH–H_2O, 30:3:1, lower layer) → MeOH] to furnish 1-deoxy-4-thio-D-arabinofuranose (**1a**, 3 mg, 44%). Compound **1a** was identified by comparison of the physical data ($[\alpha]_D$, IR, ^1H NMR, ^{13}C NMR) with authentic sample prepared from D-xylose using the previously reported method.[13]

Bioassay

Inhibitory effects on increase of serum glucose levels in oral several sugar-loaded rats. Male Wistar rats (Kiwa Laboratory Animals, Ltd., Wakayama, Japan) weighing 130–170 g were starved 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 thereafter, a water solution (5 mL/kg) of sucrose (1.0 g/kg), maltose (1.0 g/kg), or glucose (1.0 g/kg) was orally administered. Blood (ca. 0.4 mL) was collected from 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 assayed by the glucose-oxidase method (Glucose CII-test Wako, Wako Pure Industries).

Effects on serum glucose levels in alloxan-induced diabetic mice. Male ddY mice (Kiwa Laboratory Animals, Ltd., Wakayama, Japan) weighting 25–30 g were starved for 20–24 h but allowed water ad libitum. Alloxan (50 mg/kg) in saline was injected intravenously. Two days thereafter, mice were again starved for 20–24 h, and then the methanolic extract was suspended in 5% acacia solution and given orally at 10 mL/kg. Insulin was dissolved in saline and injected intraperitoneally at 10 mL/kg. Blood was collected from infraorbital venous plexus before (0 h), and 1.0 and 3.0 h after administration of test sample.

Statistics

Statistical significance of differences was estimated by analysis of variance (ANOVA) followed by Dunnett's test.[14] Values were expressed as the means ± SEM (Tables 1–3 and 7).

Glucosidase inhibitory activity. Rat small intestinal brush border membrane vesicles were prepared[15] and its suspension in 0.1 M maleate buffer (pH 6.0) was used as the small intestinal α-glucosidase of maltase, sucrase, isomaltase, and trehalase. The enzyme suspension was diluted to hydrolyse each substrate to produce D-glucose at the rate of 8.5–18.5 nmol/min/tube in the following reaction. Reaction was performed by slight modification of the procedure of Dahlqvist.[16] The substrate (maltose: 37 mM, sucrose: 37 mM, isomaltose: 3.7 mM, and trehalose 3.7 mM), test compound and the enzyme in 0.1 M maleate buffer (pH 6.0, 0.2 mL) were incubated together at 37 °C. After 30 min of incubation, 0.8 mL of water was added to the test tube, and the tube was immediately immersed in boiling water for 2 min to stop the reaction,

then cooled with water. Glucose concentration was determined by the glucose-oxidase method.

To examine the effect on β-glucosidase, β-glucosidase (0.25 U/mL, from almond, Sigma) and *p*-nitrophenyl-β-D-glucopyranoside (2.5 mM) as a substrate, and test compound in 0.4 mL acetate buffer (0.1 M, pH 5.0) were incubated. After incubation for 15 min at 37 °C and interrupting the reaction by addition of 0.4 mL 0.2 M sodium carbonate, the amount of *p*-nitrophenol was determined colorimetrically at 405 nm.

The IC_{50} value was determined graphically by a plot of percent inhibition versus log of the test compound. For kinetic analyses of maltase, sucrase, and isomaltase by salacinol (**1**), the enzyme and test samples (**1**, 1.0 μg/mL for maltase and 0.2 μg/mL for sucrase and isomaltase; acarbose, 0.5 μg/mL for maltase and sucrase and 50 μg/mL for isomaltase; voglibose, 0.1 μg/mL for maltase, 0.03 μg/mL for sucrase, and 0.25 μg/mL for isomaltase) were incubated with increasing concentrations of maltose (3.1–37 mM), sucrose (4.6–37 mM), or isomaltose (0.46–3.7 mM).

References and Notes

1. The family of *Salacia reticulata* is recently classified as Hippocrateaceae rather than Celastraceae.
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