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SALAPRINOL AND PONKORANOL WITH THIOSUGAR SULFONIUM SULFATE STRUCTURE FROM *SALACIA PRINOIDES* AND α-GLUCOSIDASE INHIBITORY ACTIVITY OF PONKORANOL AND KOTALANOL DESULFATE

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Abstract – The methanolic extract from the roots and stems of Indian *Salacia prinoides* and its water-eluted fraction of Diaion HP-20 column were found to exhibit inhibitory activities against α-glucosidase. From the water-eluted fraction, two new unique constituents with thiosugar sulfonium sulfate, salaprinol (**1**) and ponkoranol (**2**), were isolated together with 10 known constituents including salacinol and kotalanol. The structures of **1** and **2** were elucidated on the basis of chemical and physicochemical evidence. Furthermore, ponkoranol (**2**) and kotalanol desulfate (**14**) were found to show potent inhibitory activities against α-glucosidase.

In the course of our studies on antidiabetogenic compounds from natural medicines,[1-5] we have isolated two potent α-glucosidase inhibitors, salacinol (**3**) and kotalanol (**4**), with unique thiosugar sulfonium sulfate inner salt structure from the roots, stems, and leaves of *Salacia reticulata*,[6-14] *S. oblonga*,[15] and *S. chinensis*[16-20] in Sri Lanka, India, and Thailand. As a continuation of our studies on *Salacia* species plant,[21] we have found that the methanolic extract from the roots and stems of Indian *S. prinoides* exhibited inhibitory activity against α-glucosidase. This plant is called as "Kushan" in Sanskrit and is widely distributed in India, Sri Lanka, and Southeast Asia countries. The roots and stems of this plant are extensively used for the treatment of diabetes and also used as an abortifacient in Indian traditional medicine. Through bioassay-guided separation using α-glucosidase inhibitory activities, we isolated two new constituents named salaprinol (**1**) and ponkoranol (**2**) together with 10 known constituents including salacinol and kotalanol. In this paper, we describe the isolation and structure elucidation of salaprinol (**1**) and ponkoranol (**2**), as well as the inhibitory activities of **2** and related compounds on α-glucosidase.

Since the methanolic extract (3.9%) from the roots and stems of Indian *S. prinoides* exhibited inhibitory activities against α-glucosidase (Table 1), it was partitioned into an EtOAc/H_2O (1:1, v/v) mixture to furnish an EtOAc layer (2.0%) and an aqueous layer. The aqueous layer was subjected to Diaion HP-20 column chromatography (H_2O → MeOH → acetone) to give a water (H_2O)-eluted fraction (0.80%), a MeOH-eluted fraction (0.98%) and an acetone-eluted fraction (0.13%). The H_2O-eluted fraction showed potent inhibitory activity, while the EtOAc layer and the MeOH- and acetone-eluted fractions showed weak activity. The H_2O-eluted fraction was separated by NH Chromatorex column chromatography and HPLC to give salaprinol (**1**, 0.0014%), ponkoranol (**2**, 0.0034%), salacinol (**3**, 0.053%),[6–8,12] kotalanol (**4**, 0.0066%),[6–8,12] 1-deoxy-4-thio-D-arabinofuranose (**5**, 0.0021%),[12] glycerol (**6**, 0.0076%),[22] erythritol (**7**, 0.0043%),[22] D-arabinitol (**8**, 0.039%),[22] dulcitol (**9**, 0.11%),[22] D-mannitol (**10**, 0.0015%),[22] D-sorbitol (**11**, 0.0043%),[22] and *myo*-inositol (**12**, 0.00077%)[22] (Chart 1).

Table 1. Inhibitory effects of methanol-soluble part and its fractions from *S. prinoides* on α-glucosidase

	IC_{50} (μg/ml)	
	Maltase	Sucrase
MeOH extract	97	15
EtOAc layer	>200	>200
H_2O-eluted fraction	18	3
MeOH-eluted fraction	>200	107
acetone-eluted fraction	>200	>200

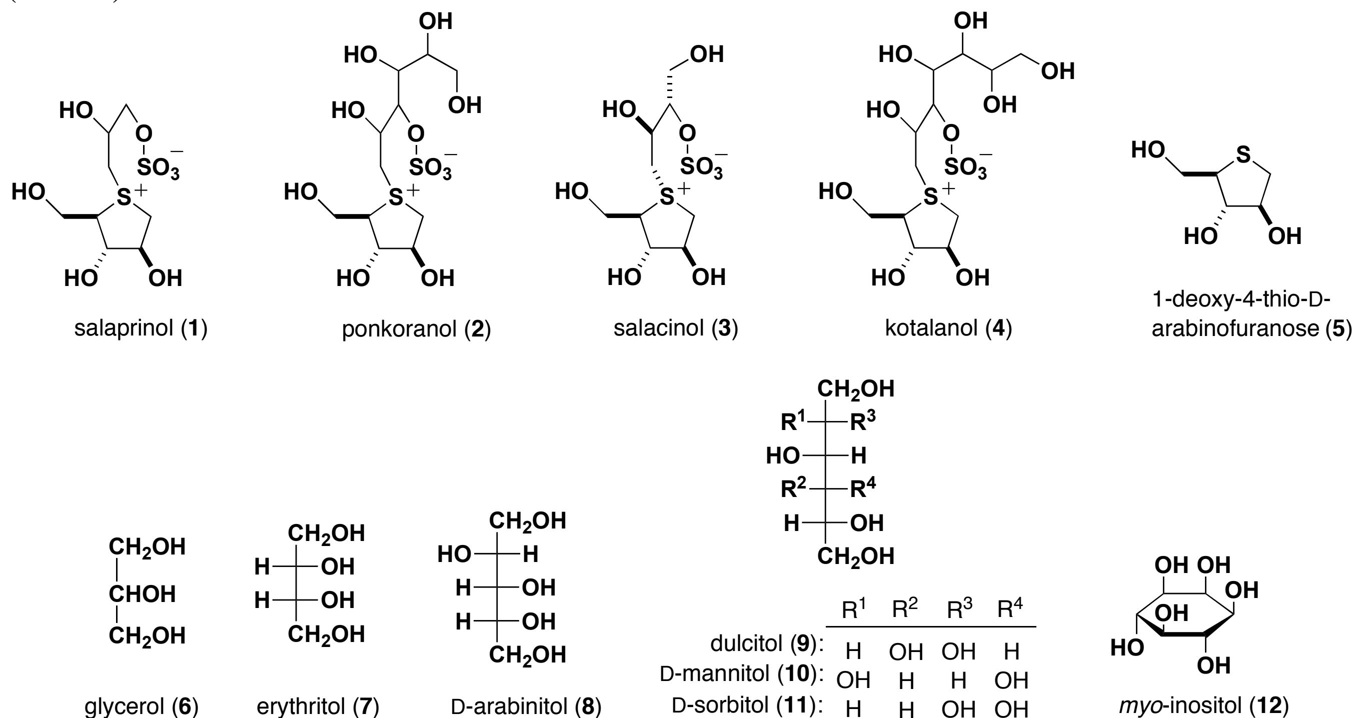

Chart 1

Structures of Salaprinol (1) and Ponkoranol (2)

Salaprinol (**1**) was isolated as a colorless powder with positive optical rotation ($[\alpha]_D^{27}$ +10.3° in MeOH). The IR spectrum of **1** showed absorption bands due to hydroxyl (3343 cm^{-1}) and sulfate (1340, 1260 cm^{-1})

functions. The FAB-MS of **1** showed quasimolecular ion peaks at *m/z* 327 $(M + Na)^+$ and *m/z* 305 $(M + H)^+$ in addition to a fragment ion peak at *m/z* 225 $(M - SO_3 + H)^+$, while a quisimolecular ion peak was observed at *m/z* 303 $(M - H)^-$ in the negative-ion FAB-MS. The molecular formula of **1** has been shown to be $C_8H_{16}O_8S_2$ by high-resolution (HR) MS analysis. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 2) spectra of **1**, which were assigned by various NMR experiment,[23] showed signals assignable to four methylenes linking to a heteroatom {δ [3.71 (1H, dd, *J* = 8.2, 13.0 Hz), 3.84 (1H, dd, *J*=3.4, 13.0 Hz), 1'-H$_2$], [3.85 (1H, dd, *J* = 3.5, 12.4 Hz), 3.88 (1H, dd, *J* = 2.0, 12.4 Hz), 1-H$_2$], [3.93 (1H, dd, *J* = 11.0, 13.0 Hz), 4.05 (1H, dd, *J* = 5.5, 13.0 Hz), 5-H$_2$], [4.00 (1H, dd, *J* = 6.2, 11.0 Hz), 4.11 (1H, dd, *J* = 4.8, 11.0 Hz), 3'-H$_2$]} and four methines bonding to a heteroatom [δ 4.04 (1H, dd like, 4-H), 4.35 (1H, m, 2'-H), 4.38 (1H, br s like, 3-H), 4.63 (1H, ddd like, 2-H)]. As shown in Figure 1, the double quantum filter correlation spectroscopy (DQF COSY) experiment on **1** indicated the presence of partial structures written in bold lines. In the heteronuclear multiple-bond correlations (HMBC) experiment, long-range correlations were observed between the following protons and carbons: 1-H and 2, 3, 1'-C; 2-H and 1, 4-C; 3-H and 1, 4, 5-C; 4-H and 2, 3, 1'-C; 5-H and 3, 4-C; 1'-H and 1, 4, 2', 3'-C; 3'-H and 1', 2'-C, so that the plane structure of **1** was clarified. Finally, alkaline treatment of **1** with 1% sodium methoxide (NaOMe)-MeOH liberated 1-deoxy-4-thio-D-arabinofuranose (**5**). Consequently, the structure of salaprinol (**1**) was determined as shown, except for the stereostructure of the 2'-position.[24]

Table 2. ^{13}C-NMR data (CD_3OD) for compound A (**1**), B (**2**)

	1	**2**
1	51.7	51.3
2	79.4	79.1
3	79.6	79.8
4	73.7	73.4
5	60.9	60.9
1'	51.3	52.7
2'	67.4	68.6
3'	70.4	81.1
4'		70.1
5'		73.2
6'		64.2

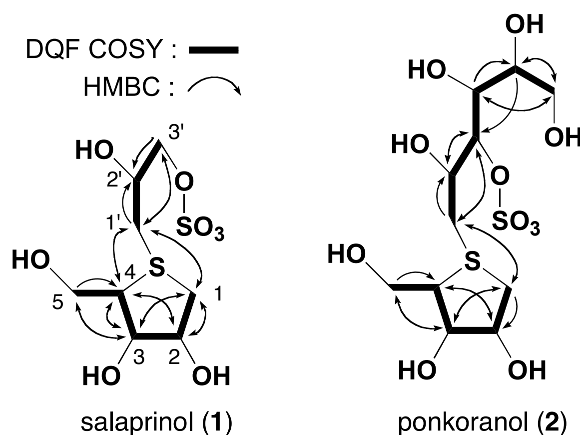

Figure 1

Ponkoranol (**2**) was also obtained as a colorless powder with positive optical rotation ($[\alpha]_D^{27}$ +13.5° in MeOH). The IR spectrum showed absorption bands at 3325, 1340, and 1265 cm^{-1} due to hydroxyl, and sulfate functions. The molecular formula, $C_{11}H_{22}O_{11}S_2$, was determined from the positive- and negative-ion FAB-MS [*m/z* 417 $(M + Na)^+$, *m/z* 393 $(M - H)^-$] and by HRFABMS measurement. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **3** and **4**, except for a part of the signals due to the side chain part. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[23] of **2** showed signals assignable to four methylenes linking to a heteroatom [δ 3.67 (2H, br d, *J* = *ca.* 2 Hz,

6'-H_2), 3.82 (2H, br s, 1-H_2), 3.93, 4.05 (1H each, both dd like, 1'-H_2), 3.97, 4.03 (1H each, both m, 5-H_2)] and seven methines bonding to a heteroatom [δ 3.90 (1H, m, 5'-H), 3.99 (1H, m, 4'-H), 4.01 (1H, m, 4-H), 4.42 (1H, m, 3-H), 4.43 (1H, m, 2'-H), 4.44 (1H, m, 3'-H), 4.61 (1H, br d, J = *ca.* 2 Hz, 2-H)]. The DQF COSY experiment on **2** showed correlations as shown in Figure 1. Furthermore, in the HMBC experiment, long-range correlations were observed between the following protons and carbons: 1-H and 2, 3, 1'-C; 2-H and 4-C; 3-H and 1, 5-C; 4-H and 2-C; 5-H and 3, 4-C; 1'-H and 1, 2', 3'-C; 2'-H and 3'-C; 3'-H and 1', 2'-C; 4'-H and 5', 6'-C; 5'-H and 3', 6'-C; 6'-H and 4', 5'-C. Finally, alkaline treatment of **2** with 1% NaOMe-MeOH liberated 1-deoxy-4-thio-D-arabinofuranose (**5**). On the basis of this evidence and comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **3** and **4**, the structure of ponkoranol (**2**) was determined as shown.[25]

α-Glucosidase Inhibitory Activity of Constituents from *S. prinoides*

The α-glucosidase inhibitory activity of salaprinol (**1**) and ponkoranol (**2**) was tested for the intestinal α-glucosidase (maltase, sucrase, and isomaltase) in vitro and compared with that of diastereomeric mixture **1'**[13] with respect to the configuration of the 2'-hydroxyl group in salaprinol (**1**), salacinol (**3**), kotalanol (**4**), and the desulfate analog (**13**)[14] of **3** (Table 3, Figure 2). In addition, the inhibitory activity of the desulfate analog (**14**) of **4**, which was prepared according to the reported method,[14] was also examined. Ponkoranol (**2**) showed potent inhibitory activity against maltase, sucrase and isomaltase. The activities of **2** were equivalent to or stronger than those of **3** and **4**, which were already known to display α-glucosidase inhibitory activities, and much stronger than that of **1** and **1'**. In addition, desulfate analog (**13**) was found to show potent inhibitory activity against isomaltase. On the other hand, the maltase and isomaltase inhibitory activities of **14** were similar to those of **3** and **4**, while the sucrase inhibitory activity was weaker than those of **3**, **4**, and **13**.

Table 3. α-Glucosidase Inhibitory Activities of Constituents (**1–4**) from *S. prinoides* and Synthetic Related Compounds (**1'**, **13**, **14**)

	IC_{50} (μM)		
	maltase	sucrase	isomaltase
salaprinol (**1**)	>100	>100	–
1'	>1320	780	–
ponkoranol (**2**)	3.2	0.29	2.6
salacinol (**3**)	5.2[a]	1.6[a]	1.3
kotalanol (**4**)	7.2[a]	0.75[a]	5.7[a]
salacinol desulfate (**13**)	8.0[a]	1.3[a]	0.30
kotalanol desulfate (**14**)	4.8	4.5	1.8

[a]These IC_{50} value were already reported by us, but the activities of these constituents were examined for comparison again.

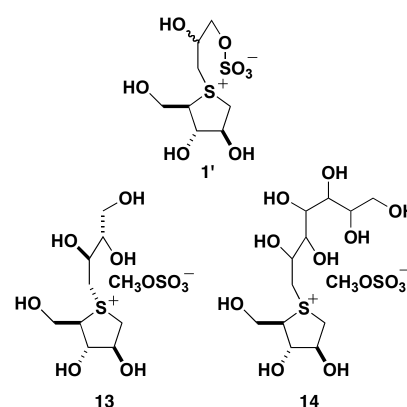

Figure 2

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; ^{1}H NMR spectra, JEOL EX-270 (270 MHz), JNM-LA500 (500 MHz), and JEOL ECA-600K (600 MHz) spectrometers; ^{13}C NMR spectra, JEOL EX-270 (68 MHz), JNM-LA500 (125 MHz), and JEOL ECA-600K (150 MHz) spectrometers with tetramethylsilane as an internal standard; EIMS and HREIMS, JEOL JMS-GCMATE mass spectrometer; FABMS and HRFABMS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV-VIS detectors. HPLC column, YMC-Pack Polyamine II (YMC Inc., 250 × 4.6 mm i.d.) and (250 × 20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

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); NH column chromatography, Chromatorex NH-DM 1020 (Fuji Silysia Chemical, Ltd., 100–200 mesh); Diaion HP-20 column chromatography (Nippon Rensui); 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); detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Plant Material

The roots and stems of *Salacia prinoides* were collected in Tamilnadu, India in 2006 and identified by one of authors (M. Y.). A voucher of the plant is on file in our laboratory (2006.salacia-06).

Extraction and Isolation

The dried roots and stems of *S. prinoides* (6.0 kg) were crushed and extracted three times with methanol (MeOH) under reflux for 3 h. Evaporation of the solvent under reduced pressure gave the methanolic extract (235.0 g, 3.9%). The MeOH extract (172.0 g) was partitioned into an EtOAc–H_2O (1:1, v/v) mixture, to furnish an EtOAc-soluble fraction (88.0 g, 2.0%) and an aqueous phase. The aqueous phase was subjected to Diaion HP-20 column chromatography (2.0 kg, H_2O → MeOH → acetone) to give H_2O-, MeOH-, and acetone-eluted fractions (35.0 g, 0.8%; 43.0 g, 0.98%; and 5.6 g, 0.13%), respectively.

The H_2O-eluted fraction (30.0 g) was subjected to NH column chromatography [0.9 kg, MeCN–H_2O (90:10 → 70:30 → 50:50, v/v) → H_2O] to give eleven fractions [Fr. 1 (0.22 g), Fr. 2 (0.99 g), Fr. 3 (3.87 g), Fr. 4 (2.15 g), Fr. 5 (1.81 g), Fr. 6 (1.70 g), Fr. 7 (5.11 g), Fr. 8 (3.26 g), Fr. 9 (0.81 g), Fr. 10 (1.78 g), and Fr. 11 (19.33 g)]. Fraction 2 (0.99 g) was purified by HPLC [YMC-Pack Polyamine II, MeCN–H_2O (90:10, v/v)] to give 1-deoxy-4-thio-D-arabinofuranose (**5**, 77.7 mg, 0.0021%) and glycerol (**6**, 287.8mg, 0.0076%). Fraction 3 (1.00 g) and Fraction 4 (1.00 g) were separated by HPLC [MeCN–H_2O (85:15, v/v)] to give erythritol (**7**, 42.3 mg, 0.0043%) and D-arabinitol (**8**, 242.4 mg, 0.014%), respectively. Fraction 5 (1.0 g) was separated by HPLC [MeCN–H_2O (85:15, v/v)] to give D-arabinitol (**8**, 389.5 mg, 0.019%), dulcitol (**9**, 260.1 mg, 0.013%), D-mannitol (**10**, 30.7 mg, 0.0015%), and D-sorbitol (**11**, 66.3 mg, 0.0032%). Fraction 6 (1.70 g) was separated by HPLC [MeCN–H_2O (80:20, v/v)] to give D-arabinitol (**8**, 46.6 mg, 0.0012%), dulcitol (**9**, 32.6 mg, 0.00087%), and D-sorbitol (**11**, 41.0 mg, 0.0011%). Fraction 7 (2.00 g) was purified by HPLC [MeCN–H_2O (80:20, v/v)] to give salacinol (**3**,

732.3 mg, 0.050%), salaprinol (**1**, 20.0 mg, 0.0014%), D-arabinitol (**8**, 51.2 mg, 0.0035%), and dulcitol (**9**, 701.7 mg, 0.048%). Fraction 8 (1.00 g) was purified by HPLC [MeCN–H_2O (80:20, v/v)] to give salacinol (**3**, 35.3 mg), D-arabinitol (**8**, 17.3 mg), and dulcitol (**9**, 491.8 mg). Fraction 9 (0.81 g) was purified by HPLC [MeCN–H_2O (75:25, v/v)] to give ponkoranol (**2**, 129.4 mg, 0.0034%), dulcitol (**9**, 19.3 mg, 0.00051%), and *myo*-inositol (**12**, 29.1 mg, 0.00077%). Fraction 10 (1.00 g) was separated by HPLC [MeCN–H_2O (75:25, v/v)] to give kotalanol (**4**, 138.6 mg, 0.0066%) and dulcitol (**9**, 28.9 mg, 0.0014%). The known compounds (**3–12**) were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR, MS) with those of authentic natural or commercial samples.

Salaprinol (**1**): A colorless powder, $[\alpha]_D^{27}$ +10.3° (*c* 1.30, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_8H_{16}O_8S_2Na$ $(M+Na)^+$: 327.0184. Found: 327.0191. IR (KBr): 3343, 1340, 1296, 1260, 1092, 1048 cm^{-1}. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 3.71 (1H, dd, *J* = 8.2, 13.0 Hz, 1'-Ha), 3.84 (1H, dd, *J*=3.4, 13.0 Hz, 1'-Hb), 3.85 (1H, dd, *J* = 3.5, 12.4 Hz, 1-Ha), 3.88 (1H, dd, *J* = 2.0, 12.4 Hz, 1-Hb), 3.93 (1H, dd, *J* = 11.0, 13.0 Hz, 5-Ha), 4.00 (1H, dd, *J* = 6.2, 11.0 Hz, 3'-Ha), 4.04 (1H, dd like, 4-H), 4.05 (1H, dd, *J* = 5.5, 13.0 Hz, 5-Hb), 4.11 (1H, dd, *J* = 4.8, 11.0 Hz, 3'-Hb), 4.35 (1H, m, 2'-H), 4.38 (1H, br s like, 3-H), 4.63 (1H, ddd like, 2-H). ^{13}C-NMR (150 MHz, CD_3OD) δ_c: given in Table 2. Positive-ion FAB-MS: *m/z* 327 $(M + Na)^+$, 305 $(M + H)^+$, *m/z* 225 $(M - SO_3 + H)^+$. Negative-ion FAB-MS: *m/z* 303 $(M - H)^-$.

Ponkoranol (**2**): A colorless powder, $[\alpha]_D^{27}$ +13.5° (*c* 1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{11}H_{22}O_{11}S_2Na$ $(M+Na)^+$: 417.0501. Found: 417.0497. IR (KBr): 3325, 1340, 1265, 1093, 1052 cm^{-1}. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 3.67 (2H, br d, *J* = *ca*. 2 Hz, 6'-H_2), 3.82 (2H, br s, 1-H_2), 3.90 (1H, m, 5'-H), 3.93, 4.05 (1H each, both dd like, 1'-H_2), 3.97, 4.03 (1H each, both m, 5-H_2), 3.99 (1H, m, 4'-H), 4.01 (1H, m, 4-H), 4.42 (1H, m, 3-H), 4.43 (1H, m, 2'-H), 4.44 (1H, m, 3'-H), 4.61 (1H, br d, *J* = *ca*. 2 Hz, 2-H). ^{13}C-NMR (150 MHz, CD_3OD) δ_c: given in Table 2. Positive-ion FAB-MS: *m/z* 417 $(M + Na)^+$. Negative-ion FAB-MS: *m/z* 393 $(M - H)^-$.

Alkaline Hydrolysis of 1 and 2

A solution of **2** (10.3 mg, 45 μmol) in 1% NaOMe–MeOH (2.0 mL) was stirred at 50 °C 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 [1.0 g, ($CHCl_3$–MeOH–H_2O, 30 : 3 : 1, lower layer) → MeOH] to furnish 1-deoxy-4-thio-D-arabinofuranose (**5**, 2.1 mg, 54%). Using a similar procedure, **5** (1.1 mg) was obtained from **1** (4.2 mg). The obtained **5** was identified by comparison of its physical data ($[\alpha]_D$, ^{1}H NMR, ^{13}C NMR, MS) with those of the isolated compound and authentic sample prepared from D-xylose using the previously reported method.[13]

Synthesis of Kotalanol Desulfate (14) from 4

A mixture of kotalanol (**4**, 10 mg, 0.024 mmol) and 5% methanolic hydrogen chloride (1.4 mL) was stirred at 45 °C for 3.5 hr. After evaporation of the reaction mixture, the residue (10.2 mg) was dissolved in MeOH (2 mL), and the resulting mixture was treated with ion exchange resin IRA-67 to give **14** (10.1 mg, 94%).

Compound **14**: A colorless oil; $[\alpha]_D^{23}$ +13.1° (*c* 0.60, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{12}H_{25}O_9S$ (M – CH_3OSO_3)$^+$: 345.1219. Found: 345.1211. IR (KBr): 3379, 1229, 1075 cm^{-1}. ^{1}H-NMR (CD_3OD, 600 MHz) δ: 3.65 (1H, dd, *J* = 1.0, 7.5 Hz, 4'-H), 3.66 (2H, d like, *J* = 6.0 Hz, 7'-H_2), 3.68 (3H, s, CH_3OSO_3), 3.76 (1H, dd, *J* = 8.5, 13.0 Hz, 1'a-H), 3.84 (1H, d like, *J* = *ca*. 8.3 Hz, 3'-H), 3.85 (1H, d like, *J* = *ca*. 7.5 Hz, 5'-H), 3.87 (2H, d like, *J* = 2.6 Hz, 1-H_2), 3.93 (1H, m, 6'-H), 3.93 (1H, dd, *J* = 8.8, 10.8 Hz, 5-Ha), 3.94 (1H, dd, *J* = 3.4, 13.0 Hz, 1'b-H), 4.02 (1H, br dd, *J* = 5.3, 8.8 Hz, 4-H), 4.05 (1H, dd, *J* = 5.3, 10.8 Hz, 5-Hb), 4.18 (1H, ddd, *J* = 3.4, 8.3, 8.5 Hz, 2'-H), 4.37 (1H, dd, *J* = 1.2, 2.4 Hz, 3-H), 4.62 (1H, ddd, *J* = 2.4, 2.6, 2.6 Hz, 2-H). ^{13}C-NMR (150 MHz, CD_3OD) δ_c 51.9 (1-C), 52.7 (1'-C), 55.2 (CH_3OSO_3), 61.1 (5-C), 65.0 (7'-C), 69.7 (2'-C), 70.2 (3'-C), 71.3 (4'-C), 71.7 (6'-C), 73.6 (5'-C), 73.7 (4-C), 79.4 (2-C), 79.5 (3-C). Positive-ion FAB-MS: *m/z* 345 (M – CH_3OSO_3)$^+$. Negative-ion FAB-MS: *m/z* 111 (CH_3OSO_3)$^-$.

Enzyme Inhibition Assays

Rat small intestinal brush border membrane vesicles were prepared and its suspension in 0.1M maleate buffer (pH 6.0) was used as small intestinal α-glucosidase of maltase and sucrase. Test compound was dissolved in dimethylsulfoxide (DMSO), and the resulting solution was diluted with 0.1M maleate buffer to prepare the test compound solution (concentration of DMSO: 10%). The substrate solution in the maleate buffer (maltose, 74 mM; sucrose, 74 mM; isomaltose, 7.4 mM, 100μL), test compound solution (50μL), and the enzyme solution (50μL) were mixed and incubated at 37 °C for 30 min. After incubation, the solution was mixed with water (0.8mL) and was immediately heated by boiling water for 2 min to stop the reaction. Glucose concentration was determined by the glucose-oxidase method. Final concentration of DMSO in test solution was 2.5% and no influence of DMSO was detected on the inhibitory activity.

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22. Those known compounds were identified by comparison of their physical data with those of commercially obtained samples.

23. The ^{1}H and ^{13}C NMR spectra of **1** and **2** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments.
24. The diastereomixture (**1'**) of the 2'-position of **1** was synthesized by us,[13] but each isomer has not been separated yet.
25. The stereostructures of the 2', 3', 4', and 5'-positions in **2** have not been characterized yet.