Pergamon

Tetrahedron Letters, Vol. 38, No. 48, pp. 8367-8370, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)10270-2

SALACINOL, POTENT ANTIDIABETIC PRINCIPLE WITH UNIQUE THIOSUGAR SULFONIUM SULFATE STRUCTURE FROM THE AYURVEDIC TRADITIONAL MEDICINE *Salacia reticulata* IN SRI LANKA AND INDIA†

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Abstract: A most potent natural α-glucosidase inhibitor named salacinol has been isolated from an antidiabetic Ayurvedic traditional medicine, *Salacia reticulata* WIGHT, through bioassay-guided separation. The stereostructure of salacinol was determined on the basis of chemical and physicochemical evidence, which included the X-ray crystallographic analysis, and the molecular conformation showed the unique spiro-like configuration of the inner salt comprised of 1-deoxy-4-thioarabinofuranosyl cation and 1'-deoxyerythrosyl-3'-sulfate anion.

Salacia reticulata WIGHT (Celastaceae) called "Kotala himbutu" in Singhalese is a woody climbing plant in the submontane forests in Sri Lanka and India. The roots and stems of this plant have been extensively used in treatment of diabetes in the Ayurvedic system of Indian traditional medicine. Previous studies on this plant have resulted in the isolation of gutta-percha, sterols, triterpenes, xanthons, and phenolic compounds,[1] but the active principle and pharmacological properties were left unclarified.

In the course of our studies on antidiabetic principles of natural medicines and medicinal foodstuffs, many triterpene oligoglycosides with inhibitory activity on the increase of serum glucose levels in glucose-loaded rats were isolated from *Aralia elata* (roots, bark, young shoots),[2] *Aesculus hippocastanum* (seeds),[3] *Polygala senega* var. *latifolia* (roots),[4] *Beta vulgaris* (roots),[5] *Gymnema sylvestre* (leaves),[6] and *Kochia scoparia* (fruit).[7] In addition, we have reported the structure requirement of saponins for inhibitory activity and the action mechanisms of the inhibitory activity.[8] As a continuing part of our screening for antidiabetic principles of natural medicine, we have found that the water-soluble fraction (25-100 mg/kg, *p.o.*) from the roots and stems of *S. reticulata* strongly inhibited the increase of serum glucose levels after the administration of sucrose or maltose, but not glucose, in rats. Furthermore, the fraction inhibited rat intestinal maltase and sucrase *in vitro* ; the IC_{50} values were 35 μg/ml to maltase (substrate : maltose 37 mM), and 26 μg/ml to sucrase (substrate : sucrose 37 mM), although the extract even at high dose did not

† Dedicated to Dr. Yoshito Kishi, Professor Harvard University, in celebration of his 60th birthday.

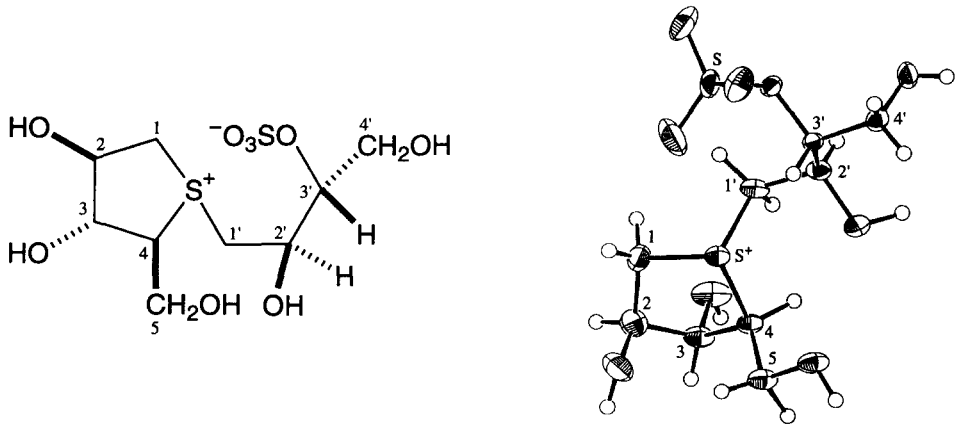

Figure 1. Computer-generated Perspective Drawing and Chemical Structure of Salacinol (**1**)

have any effect on experimental hyperglycemia induced by injection of alloxan in mice. This evidence led us to presume that the traditional antidiabetic property of this natural medicine was attributed to intestinal α-glucosidase inhibitory activity, and we undertook the bioassay-guided separation using intestinal α-glucosidase inhibitory activity to elucidate the active constituents.

Recently, intestinal α-glucosidase inhibitors were postulated to be powerful therapeutic agents in carbohydrate metabolic disorders, especially diabetes mellitus. Postprandial hyperglycemia and hyperinsulinemia are expected to be diminished by inhibition of poly- and oligosaccharide digestion in the intestinal tract. Practically, a few α-glucosidase inhibitors of microbial origin, *i.e.*, acarbose,[9] are clinically used for the treatment of diabetes mellitus.

The water-soluble fraction from the dried roots of *S. reticulata* was subjected to silica gel column chromatography and repeated various HPLC [Shodex SC1011 (Ca^{2+}), Shodex SP0810 (Pb^{2+}), and YMC-Pack Polyamine II] to give a new inhibitor named salacinol (**1**, 0.008% yield from the natural medicine). Salacinol (**1**), colorless prisms, $[\alpha]_D^{28}$ +4.9° (*c*=0.35, MeOH), suggested the presence of a sulfate group by the positive potassium rhodizonate test,[10] and also its IR spectrum showed absorption bands due to hydroxyl (3417, 1073, 1019 cm^{-1}) and sulfate (1262, 1238, 801 cm^{-1}). The molecular formula of **1** has been shown as $C_9H_{18}O_9S_2$ by high-resolution FAB-MS and SIMS analyses. Namely, the positive-ion FAB-MS and liquid SIMS of **1** showed quasimolecular ion peaks at *m/z* 335 $(M+H)^+$ and *m/z* 357 $(M+Na)^+$ in addition to a fragment ion peak at *m/z* 255 $(M-SO_3+H)^+$, while a quasimolecular ion peak was observed at *m/z* 333 $(M-H)^-$ in the negative-ion FAB-MS. The ^1H- and ^{13}C-NMR spectral data (Table 1) of **1**, which were completely assigned with the aid of ^1H-^1H and ^1H-^{13}C COSY, HOHAHA, DEPT, NOESY, and HMBC experiments, indicated the presence of the 1-deoxy-4-thiopentafuranose (1—5-C) and 1-deoxyhexitol-3-sulfate moieties (1'—4'-C). The connections of the sulfonium moiety were confirmed by the HMBC experiment, which showed long-range correlations between the following protons and carbons: 1-H_2 and 1', 4-C; 1'-H_2 and 1, 4-C; 4-H and 1, 1'-C. Finally, the stereostructure of **1** was clarified by the X-ray crystallographic analysis.

Table 1. ^{1}H- and ^{13}C-NMR Data of Salacinol (**1**) (pyridine-d_5)

	^{1}H-NMR (500 MHz)	^{13}C-NMR (125 MHz)
1	4.33 (2H, br s)	50.7
2	5.10 (1H, br s)	78.5
3	5.12 (1H, dd-like)	79.0
4	4.69 (1H, t-like)	72.6
5	4.51 (1H, dd, *J*=8.0, 11.6 Hz)	60.2
	4.54 (1H, dd, *J*=6.8, 11.6 Hz)	
1'	4.62 (1H, dd, *J*=4.2, 13.1 Hz)	52.6
	4.76 (1H, dd, *J*=4.9, 13.1 Hz)	
2'	4.99 (1H, ddd, *J*=4.2, 4.9, 7.6 Hz)	67.6
3'	5.25 (1H, ddd, *J*=3.7, 3.9, 7.6 Hz)	79.4
4'	4.37 (1H, dd, *J*=3.9, 11.6 Hz)	62.2
	4.60 (1H, dd, *J*=3.7, 11.6 Hz)	

The crystal data are as follows : monoclinic, a=6.433 (3) Å, b=12.927 (2) Å, c=8.372 (3) Å, β=93.68 (3) Å, V=694.8 (4) Å^3, space group $P2_1$, Z=2, Dc=1.598 g/cm^3, colorless prisms, 0.15 x 0.20 x 0.20 mm, μ (MoKα)=4.05 cm^{-1}, F (000)=352. The reflectional intensities of 1812 (1675 unique) within 2θ=55.0° were collected on a Rigaku AFC5R automatic four-circle diffractometer with graphite-monochromated MoKα radiation and corrected for the Lorentz and polarization factors. The 1456 independent reflections having $I>3.00\sigma(I)$ were used for the structure determination and refinement. The present discrepancy indexes R and Rw are 0.031 and 0.041, respectively. The molecular conformation is depicted in Figure 1, which showed the unique spiro-like configuration of the inner salt comprised of 1-deoxy-4-thioarabinofuranosyl sulfonium cation and 1'-deoxyerythrosyl 3'-sulfate anion.

Salacinol (**1**) showed the competitive inhibition for the intestinal α-glucosidase *in vitro*; IC_{50} values were 3.2 μg/ml to maltase, 0.84 μg/ml to sucrase, and 0.59 μg/ml to isomaltase (substrate : isomaltose 3.7 mM). The inhibitory activities of **1** against maltase and sucrase were nearly equal to those of acarbose while its inhibitory activity against isomaltase was much more potent than that of acarbose (Table 2). Furthermore, this compound (**1** : 1.3-10 mg/kg, *p.o.*) more strongly inhibited the increase of serum glucose levels in sucrose-loaded rats than acarbose (Figure 2). Therefore, it may be concluded that salacinol (**1**) is a most potent α-glucosidase inhibitor isolated from natural medicine and is a responsible constituent of the antidiabetic Ayurvedic traditional medicine "Kotala himbutu", the roots of *S. reticulata*.

Table 2. *Ki* Values of Salacinol (**1**) for Rat Small Intestinal Disaccharidase

		Ki (μg/ml)	
Substrate	*Km* (M)	Salacinol (**1**)	Acarbose
Maltose	2.7 x 10^{-3}	0.31	0.12
Sucrose	2.0 x 10^{-2}	0.32	0.37
Isomaltose	4.5 x 10^{-3}	0.47	75

Rat small intestinal brush border membrane vesicles[11] were used as the preparation of small intestinal α-glucosidase such as maltase, sucrase and isomaltase. Reaction was performed by slight modifications of the procedure of Dahlqvist.[12] The substrate (maltose : 3-37 mM, sucrose : 3-37 mM, isomaltose : 0.46-3.7 mM), test compound and enzyme in 0.1 M maleate buffer (pH 6.0) were incubated together for 30 min at 37 °C. The glucose concentration was determined by the glucose oxidase method.

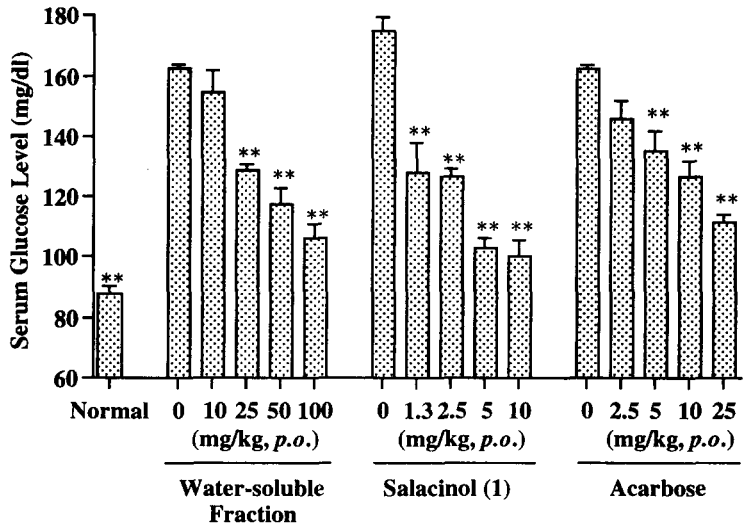

Figure 2. Inhibitory Effects of Water-soluble Fraction and Salacinol (**1**) on Increase of Serum Glucose Levels in Sucrose-loaded Rats

Male Wistar rats weighing 130-170 g were fasted for 20-24 h and the test compounds were given orally. Thirty minutes thereafter, sucrose (1 g/kg b.w.) was given orally. Each column represents the mean with S.E.M. of serum glucose levels 30 min after sucrose administration. (**p<0.01, N=5-7)

References

1) a) Kumar V., Wazeer M. I. M., Wijeratne D. B. T., *Phytochemistry*, **1985**, *24*, 2067; b) Serasinghe S., Serasinghe P., Yamazaki H., Nishiguchi K., Hombhanje F., Nakanishi S., Sawa K., Hattori M., Namba T., *Phytotherapy Res.*, **1990**, *4*, 205; c) Kumar V., Wijeratne D. B. T., Abeygunawardena C., *Phytochemistry*, **1990**, *29*, 333; d) Gunatilaka A. A. L., Dhanabalasingham B., Karunaratne V., Kikuchi T., Tezuka Y., *Tetrahedron*, **1993**, *49*, 10397; e) Tezuka Y., Kikuchi T., *J. Nat. Prod.*, **1994**, *57*, 270; f) Dhanabalasingham B., Karunaratne V., Tezuka Y., Kikuchi T., Gunatilaka A. A. L., *Phytochemistry*, **1996**, *42*, 1377.

2) a) Yoshikawa M., Yoshizumi S., Ueno T., Matsuda H., Murakami T., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **1995**, *43*, 1878; b) Yoshikawa M., Murakami T., Harada E., Murakami N., Yamahara J., Matsuda H., *ibid.*, **1996**, *44*, 1915.

3) Yoshikawa M., Murakami T., Matsuda H., Yamahara J., Murakami N., Kitagawa I., *Chem. Pharm. Bull.*, **1996**, *44*, 1454.

4) a) Yoshikawa M., Murakami T., Ueno T., Kadoya M., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **1995**, *43*, 2115; b) Yoshikawa M., Murakami T., Matsuda H., Ueno T., Kadoya M., Yamahara J., Murakami N., *ibid.*, **1996**, *44*, 1305.

5) a) Yoshikawa M., Murakami T., Kadoya M., Matsuda H., Yamahara J., Muraoka O., Murakami N., *Heterocycles*, **1995**, *41*, 1621; b) Yoshikawa M., Murakami T., Kadoya M., Matsuda H., Muraoka O., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **1996**, *44*, 1212; c) Yoshikawa M., Murakami T., Inaduki M., Hirano K., Yamahara J. Matsuda H., *ibid.*, **1997**, *45*, 561.

6) a) Murakami N., Murakami T., Kadoya M., Matsuda H., Yamahara J., Yoshikawa M., *Chem. Pharm. Bull.*, **1996**, *44*, 469; b) Yamahara J., Matsuda H., Murakami T., Shimada H., Yoshikawa M., *J. Trad. Med.*, **1996**, *13*, 295; c) Yoshikawa M., Murakami T., Kadoya M., Li Y., Murakami N., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **1997**, *45*, 1671.

7) a) Yoshikawa M., Dai Y., Shimada H., Morikawa T., Matsumura N., Yoshizumi S., Matsuda Hisa., Matsuda Hide., Kubo M., *Chem. Pharm. Bull.*, **1997**, *45*, 1052; b) Yoshikawa M., Shimada H., Murakami T., Matsumura N., Murakami T., Matsuda H., Hori K., Yamahara J., *ibid.*, **1997**, *45*, 1300.

8) Matsuda H., Murakami T., Shimada H., Matsumura N., Yoshikawa M., Yamahara J., *Biol. Pharm. Bull.*, **1997**, *20*, 717.

9) Krause H. P., Keup U., Puls W., *Digestion*, **1982**, *23*, 232.

10) a) Burma D. P., *Ana. Chim. Acta.* **1953**, *9*, 513; b) Schneider J. J., Lewbart M. L., *J. Biol. Chem.*, **1956**, *222*, 787.

11) Kessler M., Acuto O., Storelli C., Murer H., Müller M., Semenza G., *Biochim. Biophys. Acta.*, **1978**, *506*, 136.

12) Dahlqvist A., *Anal. Biochem.*, **1964**, *7*, 18.

(*Received in Japan* 2 *September* 1997; *revised* 22 *September* 1997; *accepted* 25 *September* 1997)