Antidiabetic Principles of Natural Medicines. IV.1) Aldose Reductase and α-Glucosidase Inhibitors from the Roots of *Salacia oblonga* WALL. (Celastraceae): Structure of a New Friedelane-Type Triterpene, Kotalagenin 16-Acetate

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The aqueous methanolic extract of an Indian natural medicine, the roots of *Salacia oblonga* WALL. (Celastraceae), was found to show inhibitory activity on the increase in serum glucose level in sucrose- and maltose-loaded rats. The water-soluble and ethyl acetate-soluble portions from the aqueous methanolic extract showed inhibitory activities on α-glucosidase and aldose reductase, respectively. From the water-soluble portion, potent α-glucosidase inhibitors, salacinol and kotalanol, were isolated, together with nine sugar related components, while a new friedelane-type triterpene, kotalagenin 16-acetate, was isolated from the ethyl acetate-soluble portion along with known diterpenes and triterpenes. The structure of kotalagenin 16-acetate was elucidated on the basis of physicochemical evidence. Principal components from this natural medicine were examined in terms of inhibitory activity on aldose reductase, and the diterpene and triterpene constituents, including the new kotalagenin 16-acetate, were found to be responsible components for the inhibitory activity on aldose reductase.

Key words *Salacia oblonga*; kotalagenin 16-acetate; friedelane-type triterpene; aldose reductase inhibitor; antidiabetic principle; salacinol

* Salacia (S.) oblonga WALL. (Celastraceae), called Chundan in Tamil and Ponkoranti in Malayalam, is distributed in the south region of India. The roots of *S. oblonga* have been extensively used as a remedy for gonorrhea, rheumatism, itch, asthma, and diabetes in the Ayurvedic system of traditional Indian medicine. Recently, it was reported that the rootbark powder of this natural medicine showed anti-inflammatory effects,2) but its chemical constituents including the active component, have been left uncharacterized. During the course of characterization studies on the bioactive compounds of medicinal foodstuffs3) and natural medicines,4) we have isolated two potent α-glucosidase inhibitors, salacinol (8) and kotalanol, from the stems of *Salacia reticulata* Wall. collected in Sri Lanka.5) As a continuation of our screening for antidiabetic agents in natural medicine, we have found that the methanolic extract from the roots of *S. oblonga* exhibited an inhibitory effect on the increase of serum glucose levels in sucrose- and maltose-loaded rats. The methanolic extract and its water-soluble portion showed a potent inhibitory effect on α-glucosidase, and 8 and 9 were isolated from the water-soluble portion. On the other hand, the methanolic extract and ethyl acetate-soluble portions were also found to show inhibitory activity on aldose reductase, which is related to chronic diabetic complications such as peripheral neuropathy, retinopathy, and cataracts. From the ethyl acetate-soluble portion, a new friedelane-type triterpene called kotalagenin 16-acetate (1) was isolated together with three triterpenes, two diterpenes, and catechin. This paper deals with the isolation of α-glucosidase inhibitors and the structural elucidation of a new triterpene (1). We also describe the inhibitory activity of principal components against aldose reductase.

The roots of *S. oblonga*, collected in Tamil Nade Prefecture, India, were extracted with hot aqueous methanol. The aqueous methanol extract was found to exhibit inhibitory activity on increases in serum glucose levels in sucrose (1 g/kg)- and maltose (0.5 g/kg, 1 g/kg)-loaded rats after a single oral administration of a 250 or 500 mg/kg dose, as shown in Tables 1 and 2. The extract also inhibited rat small intestinal α-glucosidase and rat lens aldose reductase (Tables 3 and 4). The methanolic extract was partitioned into an ethyl acetate–water mixture. The water-soluble portion showed potent inhibitory activity against α-glucosidase, as shown in Table 3, while the ethyl acetate-soluble portion in particular showed inhibitory activity on the aldose reductase (Table 4). The ethyl acetate-soluble portion was subjected to normal-phase and reversed-phase silica gel column chromatography, and finally HPLC, to give kotalagenin 16-acetate (1, 0.0009%), 26-hydroxy-1,3-friedelanediole (2, 0.0013%), maytenfolic acid (3, 0.0011%), salacinol (8), kotalanol (9), and high-resolution MS analysis of the quasimolecular ion peaks at m/z 291 ([M + Na]+), and 1268 ([M + 2Na–H]+, high-resolution MS analysis of the quasimolecular ion peak (M+H)+ revealed the molecular formula of 1 to be C32H50O5. The IR spectrum of 1 showed absorption bands at 3569, 1718, 1709, 1619, 1273, and 1246 cm⁻¹ assignable to hydroxyl, ketone, carbonyl, and

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olefin functions. The $^1$H-NMR (pyridine-$d_5$) and $^{13}$C-NMR (Table 5) spectra of 1, which were assigned with the aid of various NMR analytical methods, showed signals assignable to a methine bearing an acetoxyl group ($\delta$ 2.02 (s, Ac), 5.22 (dd-like, 16-H)), two isolated methylenes ($\delta$ 3.26, 3.49 (both d, $J_{15.9}$ Hz, 1-H)), 4.05, 4.20 (both d, $J_{11.5}$ Hz, 26-H), six tertiary methyls ($\delta$ 0.70, 0.94, 0.98, 1.09, 1.22, 1.38 (all s, 24, 30, 29, 27, 28, 25-H)), a secondary methyl ($\delta$ 1.04 (d, $J_{6.7}$ Hz, 23-H)), and two ketocarbonyl carbons. The planar structure of 1, including the positions of two carbonyl groups, and an acetoxyl group was clarified by a heteronuclear multiple bond correlation (HMBC) experiment on 1, which showed long-range correlations between the following protons and carbons: 2-H, 10-H and 1-C; 2-H, 4-H, 23-H, and 3-C; 28-H, and 16-C. The stereostructure of 1 was clarified by a nuclear Overhauser enhancement spectroscopy.
(NOESY) experiment, which showed nuclear Overhauser effect (NOE) correlations between the following protons (10-H and 4, 8-H; 18-H and 26-H2, 30-H 3; 26-H 2 and 25, 28-H 3; 27-H3 and 8, 16-H). This evidence and comparison of the 1H- and 13C-NMR data for 1 with those for known friedelane-type triterpenes such as 2 led us to formulate the structure of kotalagenin 16-acetate as 16-acetoxy-26-hydroxy-1,3-friedelanedione (1).

Inhibitory Effect of Constituents from the Roots of *S. oblonga* on Rat Lens Aldose Reductase  Aldose reductase as a key enzyme in the polyol pathway is 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. Since the roots of *S. oblonga* have been proven useful for the treatment of diabetes in Ayurvedic traditional medicine, we examined the inhibitory activity of its extracts (vide ante) and the components isolated from the active fractions, the ethyl acetate- and water-soluble portions, on rat lens aldose reductase. As shown in Table 6, triterpenoids (1, 3, 4) and diterpenoids (5, 6) isolated from the ethyl acetate-soluble portion showed inhibitory activity on rat lens aldose reductase, while the constituents (8—14) from the water-soluble portion exhibited little activity.

**Experimental**  The following instruments were used to obtain physical data: melting points, Yanagimoto micro-melting point apparatus MP-500D (values are uncorrected); specific rotations, Horiba SEPA-300 digital polarimeter (l=5
cm); IR spectra, Shimadzu FTIR-8100 spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer. 1H and 13C-NMR spectra, JEOL EX-270 (68 MHz) and JNM LA-500 (125 MHz) spectrometers with tetramethylsilane as an internal standard.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysa Chemical, Ltd., 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysa Chemical, Ltd., 100—200 mesh); TLC, pre-coated TLC plates with silica gel 60 F254 (Merck, 0.25 mm) (ordinary phase) and silica gel RP-18 60F254S (Merck, 0.25 mm); detection was achieved by spraying 10% methylepicatechin (515.3737. Found: 515.3744). 1H-NMR (500 MHz, CD3OD): δ 5.15 (1H, s, H-2), 4.05, 4.20 (1H each both d, J 5.15 Hz, H-3, H-4). The known compounds (2—18) were identified by comparison of their physical data (δ(29, J, 1H, 13C-NMR) with reported values 7—14) and authentic samples.

### Table 6. Inhibitory Activity of Chemical Constituents from *S. oblonga* on Rat Lens Aldose Reductase

| Table 6. Inhibitory Activity of Chemical Constituents from *S. oblonga* on Rat Lens Aldose Reductase |
| Inhibition (%) |
| Concentration (µM) | 30 | 100 |
| Kotalagenin (1-acetate) | 21.8 | 48.2 |
| 26-Hydroxy-1,3-friedelanedione (2) | 15.8 | 15.6 |
| Maytenonic acid (3) | 38.5 | 54.6 |
| 3β,22α-Dihydroxyolean-12-en-29-oic acid (4) | 55.9 | 75.9 |
| 19-Hydroxyferruginol (5) | 21.4 | 39.1 |
| Liberic acid (6) | 17.7 | 35.2 |
| 3′-O-Methylglycalactonate (7) | 11.0 | 6.9 |
| Salacinol (8) | -1.2 | -3.6 |
| Kotalanol (9) | -2.4 | 0.0 |
| Galactinol (11) | -1.3 | 11.3 |
| 3-O-α-L-Rhamnopyranosyl(1→6)-O-β-D-glucopyranosyl-sn-glycero(12) | 4.0 | 12.3 |
| Raffinose (13) | 20.0 | 11.3 |
| Stachyose (14) | -2.0 | 14.7 |

The H2O extract (50 mg) was treated with MeOH, and the suspension was filtered to remove the MeOH-insoluble portion (8.6 g, 1.1%). After removal of the solvent under reduced pressure, the MeOH-soluble portion (41 g, 5.5%) was obtained. The MeOH-soluble portion (31 g) was subjected to normal-phase silica gel column chromatography [1.5 kg, CHCl3–MeOH–H2O (6:4:1, v/v)] to give α-tocopherol–acetone–H2O (1:1, v/v) to give eight fractions [fr. 1 (0.2 g, 0.30%), fr. 2 (0.1 g, 0.20%), fr. 3 (1.0 g, 0.41%), fr. 4 (0.5 g, 0.61%), fr. 5 (0.8 g, 0.75%), fr. 6 (1.3 g, 2.39%), fr. 7 (1.0 g, 1.86%), fr. 8 (2.0 g, 3.48%), fr. 9 (1.0 g, 2.08%), fr. 10 (0.5 g, 0.98%), fr. 11 (0.5 g, 1.02%), fr. 12 (0.3 g, 0.60%), fr. 13 (0.5 g, 1.03%), fr. 14 (0.1 g, 0.20%), fr. 15 (0.3 g, 0.57%). Fraction 4 (5 g) was purified by reversed-phase silica gel chromatography [80 g, H2O–MeOH–H2O (90:5:5, v/v)] to give kotalagenin 16-acetate (1.6 g, 0.082%), fr. 7 (0.83 g, 0.40%), fr. 8 (1.1 g, 0.51%), fr. 9 (0.7 g, 0.36%), fr. 10 (0.4 g, 0.21%), fr. 11 (0.3 g, 0.17%). Fraction 5 (2.5 g) was purified by HPLC [MeOH–H2O (85:15, v/v)] to give kotalaginol (0.88 g, 0.00%), fr. 12 (0.5 g, 0.31%), fr. 13 (0.3 g, 0.19%), fr. 14 (0.2 g, 0.10%). Fraction 6 (2.4 g) was purified by reversed-phase silica gel chromatography [2 g, NH Chromatorex, CH3CN–H2O (40:60, v/v)] to give kotalanol (0.87 g, 0.11%), fr. 15 (0.5 g, 0.08%). Fraction 7 (2.9 g) was purified by reversed-phase silica gel chromatography [2 g, NH Chromatorex, CH3CN–H2O (70:30, v/v)] to give MeOH–H2O (90:10, v/v) and HPLC [YMC-Pack Polyamide II (250×20 mm i.d.), CH3CN–H2O (70:30, v/v)] to give stachyose (0.8 g, 0.09%), fr. 16 (0.6 g, 0.08%), fr. 17 (0.4 g, 0.06%), fr. 18 (0.2 g, 0.03%), fr. 19 (0.1 g, 0.01%). Fraction 8 (1.5 g) was purified by reversed-phase silica gel chromatography [50 g, H2O–MeOH] to give 19-hydroxyferruginol (0.87 g, 0.11%), fr. 20 (0.6 g, 0.08%), fr. 21 (0.4 g, 0.06%), fr. 22 (0.3 g, 0.05%). Fraction 9 (2.0 g) was purified by reversed-phase silica gel chromatography [50 g, H2O–MeOH] to give stachylose (0.8 g, 0.08%), fr. 23 (0.6 g, 0.09%), fr. 24 (0.4 g, 0.06%). Fraction 10 (2.0 g) was purified by reversed-phase silica gel chromatography [50 g, H2O–MeOH] to give stachyose (0.8 g, 0.08%), fr. 25 (0.6 g, 0.09%), fr. 26 (0.4 g, 0.06%). Fraction 11 (2.0 g) was purified by reversed-phase silica gel chromatography [50 g, H2O–MeOH] to give stachyose (0.8 g, 0.08%), fr. 27 (0.6 g, 0.09%), fr. 28 (0.4 g, 0.06%). Fraction 12 (2.0 g) was purified by reversed-phase silica gel chromatography [50 g, H2O–MeOH] to give stachyose (0.8 g, 0.08%), fr. 29 (0.6 g, 0.09%), fr. 30 (0.4 g, 0.06%). Fraction 13 (2.0 g) was purified by reversed-phase silica gel chromatography [50 g, H2O–MeOH] to give stachyose (0.8 g, 0.08%), fr. 31 (0.6 g, 0.09%), fr. 32 (0.4 g, 0.06%). Fraction 14 (2.0 g) was purified by reversed-phase silica gel chromatography [50 g, H2O–MeOH] to give stachyose (0.8 g, 0.08%), fr. 33 (0.6 g, 0.09%), fr. 34 (0.4 g, 0.06%). Fraction 15 (2.0 g) was purified by reversed-phase silica gel chromatography [50 g, H2O–MeOH] to give stachyose (0.8 g, 0.08%), fr. 35 (0.6 g, 0.09%), fr. 36 (0.4 g, 0.06%). Fraction 16 (2.0 g) was purified by reversed-phase silica gel chromatography [50 g, H2O–MeOH] to give stachyose (0.8 g, 0.08%), fr. 37 (0.6 g, 0.09%), fr. 38 (0.4 g, 0.06%). Fraction 17 (2.0 g) was purified by reversed-phase silica gel chromatography [50 g, H2O–MeOH] to give stachyose (0.8 g, 0.08%), fr. 39 (0.6 g, 0.09%), fr. 40 (0.4 g, 0.06%). Fraction 18 (2.0 g) was purified by reversed-phase silica gel chromatography [50 g, H2O–MeOH] to give stachyose (0.8 g, 0.08%), fr. 41 (0.6 g, 0.09%), fr. 42 (0.4 g, 0.06%).
modification of the procedure of Dahlqvist.  \(^{18}\) The substrate (maltose, 37 mM; sucrose, 37 mM), test compound and the enzyme in 0.1 M maleate buffer (pH 7.0) 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, then cooled with water. Glucose concentration was determined by the glucose oxidase method.

Aldose Reductase Inhibitory Activity: Aldose reductase activity was assayed according to the method described by Dufrane et al.\(^{19}\) with slight modifications. Lenses of Wistar rats were homogenized in 135 mM Na, K-phosphate buffer (pH 7.0), 100 mM Li\(_2\)SO\(_4\), 0.03 mM NADPH, 1 mM DL-glyceraldehyde as a substrate, and 100 \(\mu\)l of the enzyme fraction, with or without 25 \(\mu\)l of sample solution in a total volume of 0.5 ml. Test samples were dissolved in dimethyl sulfoxide. The reaction was initiated by the addition of NADP to a fluorescent product. The reaction was stopped by the addition of NADPH at 30 °C. After 30 min of incubation, the fluorescence was measured at room temperature using a spectrofluorometer (Type 650-10, Hitachi, Japan) with an excitation wavelength of 360 nm, and an emission wavelength of 460 nm.

References and Notes
15) The \(^1H\)- and \(^13C\)-NMR spectra were assigned on the basis of homonuclear multiple bond correlation (HMBC) experiments.