Studies on Hindered Phenols and Analogues. V.[1] Synthesis, Identification, and Antidiabetic Activity of the Glucuronide of CS-045

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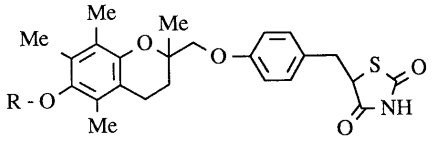
The glucuronide of a new oral antidiabetic agent, (±)-5-[4-(6-hydroxy-2,5,7,8-tetramethylchroman-2-ylmethoxy)benzyl]thiazolidine-2,4-dione (CS-045) (1), was synthesized to confirm the structure of a metabolite in monkeys (*Macaca fascicularis*) and to examine its antidiabetic activity. The glucuronide also had antidiabetic activity in KK-mice.

Keywords glucuronide; CS-045; antidiabetic activity; hindered phenol; *Macaca fascicularis*; KK-mice

(±)-5-[4-(6-Hydroxy-2,5,7,8-tetramethylchroman-2-ylmethoxy)benzyl]thiazolidine-2,4-dione (**1**, CS-045) is undergoing clinical trial (phase II study)[2] as a new oral antidiabetic agent.[3] This compound **1** is not only effective in insulin resistant diabetic animals such as the KK-mouse, ob/ob mouse, and Zucker fatty rat,[4] but also lowers lipid peroxide (LPO) which is thought to be one of the causes of macro- and microangiopathy.[3]

It has been well known that phenolic compounds can be metabolized to give the corresponding glucuronide as exemplified in Chart 2.[5] The hindered phenolic compound **1** also appeared to be metabolized to the corresponding glucuronide. We actually isolated a metabolite from the bile of a monkey (*Macaca fascicularis*), whose mass spectral data indicated the structure of the glucuronide **2**.[6]



1: R=H CS-045

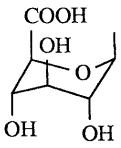
2: R= 

Chart 1

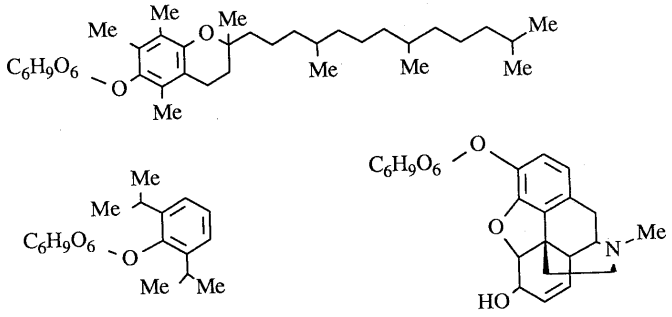


Chart 2. Examples of Phenolic Glucuronides

The presence of the glucuronide **2** might contribute to the long duration of antidiabetic activity based on so-called enterohepatic recirculation accompanying the hydrolysis of glucuronide **2**, giving the parent drug **1** in the intestine.

In this paper, we wish to report the chemical synthesis of the glucuronide **2**, its identification with the monkey metabolite isolated from bile, and the oral antidiabetic activity of **2** together with the synthetic precursor **3** in KK-mice.

Synthesis Compound **2** was synthesized by a method similar to that previously reported.[7] A mixture of compound **1** and freshly prepared methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate (**4**)[8] in nitrobenzene was heated under reduced pressure in the presence of an acid catalyst to give the corresponding protected glucuronide **3** in a 23% yield. Deprotection of compound **3** by alkaline hydrolysis gave the desired glucuronide **2** in a moderate yield.

Identification The glucuronide isolated from the bile of *M. fascicularis* was identified with the authentic glucuronide **2**, based on fast atom bombardment (FAB) mass spectrum and high performance liquid chromatography (HPLC) analysis. Also, both glucuronides gave compound **1** quantitatively by treatment with β-glucuronidase originated from *Escherichia coli*.

Antidiabetic Activity According to a manner similar to that described in reference 4, the antidiabetic activity was evaluated by the percent decrease of the serum glucose level when the test compounds were administered orally at a

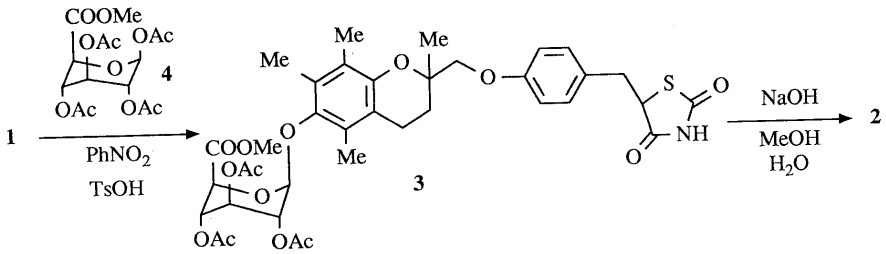


Chart 3

single dose of 50 mg/kg to genetically diabetic male KK-mice under a fed condition, which is a model animal of non-insulin dependent diabetes mellitus (NIDDM). Glucuronides **2** and **3** respectively showed 26 and 29% decreases from the control group, and these values were smaller than compound **1** which had a value of 38.5% ($p<0.05$).

Experimental

FAB mass spectra were recorded on a JEOL JMS-HX100 mass spectrometer with a 3-nitrobenzyl alcohol matrix. Proton-nuclear magnetic resonance (^{1}H-NMR) spectrum was recorded on a 500 MHz JEOL JNM-GX500 FT NMR spectrometer with tetramethylsilane as an internal standard. The abbreviation "nd" indicates that precise identification of the signal was not possible because of overlap by other signals.

Isolation of the Metabolic Glucuronide 2 from the Bile CS-045 (**1**) was administered to a bile duct cannulated monkey (*M. fascicularis*) as an infusion injection from the great saphenous vein at a dose of 5 mg/kg. During 2 h of infusion, bile was collected under anesthesia. Tweny-four ml of bile was applied to the isolation: Each 4-ml portion of bile was charged into a Sep-pak C_{18}^{TM} cartridge (Millipore, U.S.A.). Then the cartridge was washed with 10 ml of water and 2 ml of 5% methanol successively, and the objective fraction was eluted by 4 ml of 25% acetonitrile. The combined fraction was freeze-dried, and the residue was reconstituted with 10 ml of benzene–methanol (3 : 1). The solution was passed through a Sep-pak Florisil™ cartridge to remove polar contaminants. After evaporation of the solvent, the residue was reconstituted with a small amount of acetonitrile and charged into a Sep-pak C_{18} column again, to remove inorganic contaminants from Florisil. After washing with water and 5% methanol in the same manner, glucuronide **2** was eluted by 4 ml of 100% acetonitrile. The eluent was evaporated to dryness. After the residue was dissolved in a small amount of methanol, water was added until the solution became turbid. White powdered glucuronide **2** was collected by filtration after standing overnight in a refrigerator.

Methyl 2,3,4-Tri-*O*-acetyl-1-*O*-[2,[4-(2,4-dioxothiazolidin-5-ylmethyl)-phenoxymethyl]-2,5,7,8-tetramethylchroman-6-yl]-β-D-glucopyranuronate (3) A mixture of 31.0 g of (±)-5-[4-(6-hydroxy-2,5,7,8-tetramethylchroman-2-ylmethoxy)benzyl]thiazolidine-2,4-dione (**1**), 9.8 g of methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate (**4**), 1.0 g of *p*-toluenesulfonic acid monohydrate, and 87 ml of nitrobenzene was heated at 85 °C for 4 h at 20 mmHg. The reaction mixture was subjected to silica gel column chromatogaphy [eluent; benzene : ethyl acetate = 1 : 0 to 3 : 1], to give 4.6 g (23%) of caramel-like compound **3**. FAB MS *m/z*: 757 ($M^+\cdot$). ^{1}H-NMR ($CDCl_3$) δ: 1.40 (3H, s, 2-Me [chroman]), 1.82—1.96 (1H, m, 3-H [chroman]), 2.0—2.2 (1H, nd, 3-H [chroman]), 2.02 (3H, s, OAc), 2.05 (3H, s, OAc), 2.06 (3H, s, Ar-Me), 2.109 and 2.111 (total 3H, each s, OAc), 2.14 (3H, s, Ar-Me), 2.17 (3H, s, Ar-Me), 3.11 (1H, dd, J=14.3, 9.5 Hz, $C_6H_4C\underline{H}_2$), 3.44 (1H, dd, J=14.3, 4.0 Hz, $C_6H_4C\underline{H}_2$), 3.68 and 3.69 (total 3H, each s, COOMe), 3.85 (1H, d, J=9.8 Hz, 5-CH [sugar]), 3.88, 3.95 (each 1H, each d, J=9.2 Hz, $C\underline{H}_2OC_6H_4$), 4.49 (1H, dd, J=9.5, 4.0 Hz, 5-CH [thiazolidine]), 4.75 (1H, d, J=7.7 Hz, 1-CH [sugar]), 5.24 (1H, dd, J=9.8, 8.8 Hz, 4-CH [sugar]), 5.32 (1H, dd, J=9.6, 8.8 Hz, 3-CH [sugar]), 5.34 (1H, dd, J=9.6, 7.7 Hz, 2-CH [sugar]), 6.87, 7.13 (each 2H, each d, J=8.6 Hz, Ar-H), 7.98 (1H, br s, NH).[9]

1-*O*-[2-[4-(2,4-Dioxothiazolidin-5-ylmethyl)phenoxymethyl]-2,5,7,8-tetramethylchroman-6-yl]-β-D-glucopyranuronic Acid (2) To a solution of 3.9 g of **3** in 150 ml of methanol was added, dropwise, 20.63 g of 2.4% aq. sodium hydroxide solution under ice cooling. After stirring at room temperature for 1 h, dry carbon dioxide gas was bubbled into the reaction mixture. The solvent was removed under reduced pressure, the resulting residue was subjected to silica gel column chromatography [eluent; ethyl acetate : acetic acid = 5 : 1], and the residual substance was dissolved in ethyl acetate. The solution was washed with water and dried over sodium sulfate, and the solvent was removed under reduced pressure, to give 2.4 g of a pale yellow caramel-like substance. The caramel was triturated with water to give a paste, which was freeze-dried to give 1.85 g of compound **2** as a pale yellow powder. The powder was identical with the authentic metabolite from the data on the retention time of HPLC carried out with a reverse-phase column (YMC A-312 (ODS), Yamamura Chemical Laboratories Co., Ltd.) using an acetonitrile–distilled water–phosphoric acid (42 : 58 : 0.05, v/v) mixture. The powder was treated with β-glucuronidase as follows: about 1 mg of the powder was dissolved in 10 ml of a 0.1 M acetate buffer (pH 4) and the solution was treated with 260000 units of β-glucuronidase that originated from *E. coli.* at 37 °C for 2 h to give quantitatively the compound **1**, as detected by HPLC analysis with a reverse-phase column (YMC A-314) using an acetonitrile–distilled water–phosphoric acid (62 : 38 : 0.05, v/v) mixture. Compound **2**: FAB MS *m/z*: 616 $(M-H)^-$, 618 $(M+H)^+$. *Anal.* Calcd for $C_{30}H_{35}NO_{11}S\cdot 1.5H_2O$: C, 55.89; H, 5.94; N, 2.17; S. 4.97. Found: C, 56.02; H, 5.82; N, 2.19; S, 4.73.

References and Notes

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