Baicalein, an α-Glucosidase Inhibitor from *Scutellaria baicalensis*

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Methanol extracts of *Scutellaria baicalensis*, *Rheum officinale*, and *Paeonia suffruticosa* showed potent inhibitory activity against rat intestinal sucrase. The active principles were identified as baicalein from the first and methyl gallate from the last two plants. In addition to its activity against the rat enzyme, baicalein also inhibited human intestinal sucrase expressed in Caco-2 cells.

The enzyme α-glucosidase catalyzes the final step in the digestive process of carbohydrates, and hence α-glucosidase inhibitors could retard the absorption of dietary carbohydrates to suppress postprandial hyperglycemia. In fact, a potent α-glucosidase inhibitor, acarbose, has been shown to reduce effectively the intestinal absorption of sugars in humans.[1] In the course of our search for physiologically active natural substances, we have isolated and identified sulfolipids, quercetin, and *N*-*p*-coumaroyltyramine from hijiki,[2] tochu-cha,[3] and Welsh onion,[4] respectively, as α-glucosidase inhibitors. In this paper, we present results of a screen of traditional Chinese medicines for α-glucosidase inhibition.

In the screening experiments for rat intestinal sucrase inhibitors in traditional Chinese herbal medicines, 27 materials were tested. Inhibitory activities in methanol extracts (medicinal part, % inhibition) were as follows: *Gardenia jasminoides* (fruit, 4), *Panax ginseng* (root, 4), *Pachyma hoelen* (fruiting body, 0), *Atractylodes chinensis* (rhizome, 19), *Carthamus tinctorius* (flower, 5), *Coptis chinensis* (rhizome, 9), *Cinnamomum cassia* (bark, 17), *Zizyphus jujuba* (fruit, 7), *Rehmannia glutinosa* (bulb, 16), *Scrophularia buergeriana* (root, 22), *Pinellia ternata* (corn, 3), *Scutellaria baicalensis* (root, 70), *Zingiber officinale* (rhizome, 26), *Bupleurum chinense* (root, 9), *Angelica acutiloba* (root, 12), *Rheum officinale* (rhizome, 90), *Cocculus trilobus* (rhizome,12), *Prunus armeniaca* (seed, 0), *Crataegus cuneate* (fruit, 3), *Prunus persica* (seed, 15), *Paeonia lactiflora* (root, 30), *Paeonia suffruticosa* (root-bark, 96), *Ephedra equisetina* (stem, 25), *Astragalus membranaceus* (root, 9), *Pueraria lobata* (root, 0), *Glycyrrhiza glabra* (root, 17), and *Dioscorea batatas* (tuber, 1).

Because the methanol extracts of the medicinal parts of "ougon" (*Scutellaria baicalensis*), "daiou" (*Rheum officinale*), and "botanpi" (*Paeonia suffruticosa*) showed potent inhibitory activity, these extracts were subjected to phytochemical isolation. Chromatographic separation of the extracts of *R. officinale* Baill. (Polygonaceae) and *P. suffruticosa* Andr. (Paeoniaceae) resulted in the isolation of methyl gallate (**1**) as the inhibitor, whereas the *S. baicalensis* Georgi (Lamiaceae) extract gave baicalein (5,6,7-trihydroxyflavone, **2**) as an active principle. The physicochemical properties of **1** and **2** coincided with those of commercially available methyl gallate and baicalein, respectively.

It should be noted that the term sucrase in this text means a crude α-glucosidase complex that can catalyze the hydrolysis of sucrose. The sucrase inhibition was assessed by a decrease of glucose production through the enzyme reaction, and the glucose content of the reaction mixture was determined by the glucose oxidase method. Phenolic compounds such as **1** and **2**, however, may have prevented the glucose oxidase reaction and, as a consequence, lowered the apparent glucose content produced in the sucrase reaction. The exact inhibitory activity of the isolates was then re-determined by a slightly modified procedure in which the sucrase reaction mixtures containing phenolic inhibitors were passed through a short column of basic alumina prior to determination of the glucose content by the glucose oxidase method. This basic alumina pretreatment effectively removed phenolic compounds from the reaction mixture without affecting its glucose content. As a result, the inhibitory activity of methyl gallate (**1**) almost disappeared after the basic alumina treatment, whereas that of baicalein (**2**) remained unchanged. Hence, the apparent inhibitory activity of **1** is derived from the inhibition of the glucose oxidase reaction rather than that of sucrase. The 50% inhibitory concentration (IC_{50}) of **2** against rat intestinal sucrase was as high as 2.6×10^{-4} M, as compared with the positive control, 1-deoxynojirimycin (100% inhibition at 2.1×10^{-5} M).

From a viewpoint of sugar uptake suppression, it would be interesting to know if baicalein (**2**) shows an inhibitory activity against human intestinal α-glucosidase. Therefore, we examined the inhibitory activity of **2** against human enzyme by using a Caco-2 cell line, which is derived from human colon adenocarcinoma and differentiated in vitro into small-intestinal enterocyte-like cells.[5,6] The Caco-2 cells were cultivated on permeable supports from 0 to 14 days after plating to perform a sucrase assay. The activity of 1-deoxynojirimycin (positive control), *N*-*p*-coumaroyltyramine (**3**), and (+)-catechin (**4**) were tested along with **2** for comparison purposes. *N*-*p*-Coumaroyltyramine (**3**) was previously isolated as a yeast α-glucosidase inhibitor.[4] The activity of **3** against human enzyme was hence interesting, although it showed little activity against a rat enzyme.[4] (+)-Catechin (**4**) was also assayed as a phenolic structural analogue of **2**, inasmuch as it seems natural that phenolic hydroxyl groups of compounds such as **2** interact nonspecifically with the enzyme protein to inhibit its enzyme activity. Figure 1 shows the inhibitory activity of these compounds against sucrase, which is located on the mucosal side of membranous cells. Baicalein showed distinct inhibitory activity (IC_{50} 3.5×10^{-5} M), although its activity was weaker than that of 1-deoxynojirimycin (95% inhibition at 2.5×10^{-5} M). In contrast, **3** showed negligible activity against the enzyme at $\leq 1.0 \times 10^{-4}$ M. We have already demonstrated that this phenolic amide did not inhibit rat intestinal sucrase, which might be due

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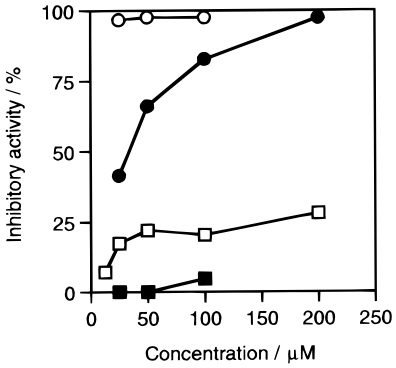
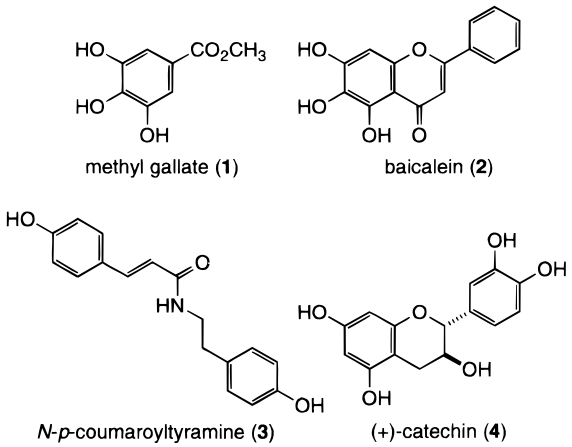


Figure 1. Inhibitory activity of compounds against Caco-2 sucrase. The activity was determined by a decrease of glucose produced in the mucosal media after incubation with sucrose. ○ = 1-deoxynojirimycin (positive control); ● = baicalein; □ = (+)-catechin; ■ = *N*-*p*-coumaroyltyramine.

to considerable structural differences in yeast and animal enzymes.[4] In addition, **4** showed only a weak inhibitory activity even though it carried four phenolic hydroxyl groups. From these results, it is suggested that the presence of phenolic hydroxyl groups themselves is not sufficient to support the inhibitory activity against the human intestinal sucrase.

The effect of **2** on the glucose transport from the mucosal to the basolateral side through Caco-2 cells was also determined in an experiment in which a glucose solution was added to the mucosal side of the cells with or without **2**. Quantitative analysis of the glucose content in the basolateral side showed no significant inhibition against glucose transport across the cells by **2** (90% of the control). Thus, it has been clearly shown that **2** inhibits only sucrase activity on the mucosal side of the Caco-2 cells and has no effect upon glucose transport. Consequently, the extract of *S. baicalensis* may be useful for suppressing postprandial hyperglycemia and for avoiding obesity arising from an excess sugar consumption.



Experimental Section

General Experimental Procedures. ^{1}H NMR spectra were measured with a Bruker AM 500 spectrometer. Chemical shifts (δ) in ppm were determined relative to residual signals of Me_2CO-d_6, δ_H 2.04. MS and IR spectra were obtained on JEOL AX500 and Perkin−Elmer 2000 instruments, respectively.

Materials. Japanese pharmacopoeial Chinese herbal medicines were purchased from Tochimoto-Tenkaido Co. (Osaka, Japan). Authentic methyl gallate and baicalein were obtained from Wako Pure Chemical Industry (Osaka, Japan), and 1-deoxynojirimycin, from Sigma Aldrich Japan Co. (Tokyo, Japan). All chemicals used were of reagent grade.

Isolation of Methyl Gallate (1). Dried medicinal parts (10 g) of *R. officinale* (daiou) were extracted with 50% aqueous MeOH (100 mL) at room temperature. The extracts were concentrated under reduced pressure and partitioned between EtOAc and H_2O. The EtOAc layer was then washed with 5% aqueous $NaHCO_3$ solution and chromatographed on Si gel with a hexane−EtOAc gradient. The fractionation was guided by the inhibitory test against rat intestinal sucrase. The active fraction, eluted with EtOAc, was subjected to Si gel preparative TLC developed with $CHCl_3$−MeOH (95:5). The active fraction of $R_f = 0.19$ obtained was purified by HPLC [column: Inertsil PREP−ODS, 6.0 × 250 mm; mobile phase MeOH−H_2O (35:65); flow rate 1.2 mL/min; detection UV 254 nm] to give methyl gallate (**1**, 5.0 mg) as an active principle, which was identified by direct comparison of its physicochemical data with an authentic sample.

Compound 1: white amorphous powder; EIMS *m*/*z* (%) 184 (M^+, 73), 153 (100), 125 (22); ^{1}H NMR δ (Me_2CO-d_6) 3.77 (3H, s, 1′-H_3), 7.09 (2H, s, 2, 6-H); IR ν_{max} (KBr) cm^{-1}: 3480, 1700. Methyl gallate was also obtained as an active principle from *P. suffruticosa* by the same procedure.

Isolation of Baicalein (2). Dried medicinal parts of *Scutellaria baicalensis* (10 g) were extracted with 50% aqueous MeOH (200 mL) at room temperature. The extracts were fractionated by the same procedure as above [except for the R_f value in the preparative TLC, 0.35, and the mobile phase of the HPLC, MeOH−H_2O (7:3)] to give baicalein (**2**, 5.0 mg) as a principal active substance, which was identified by direct comparison of its physicochemical data with the authentic sample.

Compound 2: yellow amorphous powder; EIMS *m*/*z* (%) 270 (M^+, 100), 168 (25), 107 (13); ^{1}H NMR δ (Me_2CO-d_6) 6.68 and 6.76 (each 1H, s, 3-H and 8-H), 7.26−7.62 (3H, m, 3′-, 4′-, 5′-H), 8.06 (2H, dd, J = 7.7, 1.5, 2′-, 6′-H); IR ν_{max} (KBr) cm^{-1} 3410, 1650.

Rat Intestinal Sucrase Inhibitory Activity. Crude rat small intestinal α-glucosidase complex was prepared from rat intestinal mucosa. The scraped mucosa (24 mL from 20 male rats) was homogenized in 0.1 M potassium phosphate buffer (pH 7.0) containing 5 mM EDTA at 4 °C. The precipitates obtained by centrifugation (21 000 g, 60 min) of the homogenate were solubilized in the buffer containing 1.0% Triton X-100 at 4 °C for 60 min. The solution was then centrifuged (110 000 g, 90 min), and the supernatant was dialyzed against 0.01 M potassium phosphate buffer (pH 7.0) for 24 h. The crude α-glucosidase solution contained 798 mg of protein, and its relative sucrase activity was 0.11 unit/mg protein.

Sucrase reaction mixture consisted of crude enzyme solution (0.2 mL), 28 mM sucrose in 0.1 M potassium phosphate buffer (pH 6.3) (0.5 mL) and test sample in 50% aqueous dimethyl sulfoxide (DMSO) (0.3 mL). After incubation for 15 min at 37 °C, the reaction was stopped by adding 2.0 mL of 2 M Tris-HCl buffer (pH 7.0). The amount of liberated glucose was determined by the glucose oxidase method using a commercial test kit (Glucose B test Wako, Wako Pure Chemical Industry, Osaka, Japan).

Basic alumina pretreatment of the glucosidase reactants prior to determination of the glucose content by the glucose oxidase method was carried out by passing the reaction mixture through a short column of basic alumina (ICN Alumina B, grade I, ICN Biomedicals GmbH, Eschwege, Germany).

Caco-2 Cell Assay. Caco-2 cell experiments were performed basically as reported.[7,8] Both mucosal and basolateral faces of Caco-2 cell monolayers obtained 14−15 days after seeding cells on polycarbonate membrane in a 24-well plate were washed three times with 2 mL each of phosphate-buffered saline (PBS) to remove glucose. Then a mixture of 950 μL of PBS containing 28 mM sucrose as a substrate and a test sample solution (50 μL in DMSO) were added to the mucosal side of the Caco-2 cell membrane, whereas 1 mL of PBS was added to the basolateral side. The assay plate was incubated at 37 °C for 2 h. After incubation, 100 μL each of the bathing

solution of both sides was transferred to a microtiter plate, and the content of free glucose was determined as mentioned above.

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