Ellagitannins from *Lagerstroemia speciosa* as Activators of Glucose Transport in Fat Cells

Takeo Hayashi[1], Haruko Maruyama[1], Royji Kasai[1], Katsuji Hattori[1], Shunsuke Takasuga[1], Osamu Hazeki[1], Kazuo Yamasaki[1], Takashi Tanaka[2]

Abstract

Glucose transport enhancers were searched for in *Lagerstroemia speciosa*, a Philippine local herbal medicine used for diabetes mellitus. Bioassay-guided fractionation of the aqueous acetone extract of the leaves afforded three active ellagitannins, lagerstroemin, flosin B and reginin A, identified by NMR and optical rotation. These compounds increased glucose uptake of rat adipocytes, and could be responsible for lowering the blood glucose level.

In the Philippines, *Lagerstroemia speciosa* (L.) Pers. (Lythraceae) is called "banaba", and the tea from the leaves has been used as a beverage as well as for treatment and prevention of diabetes mellitus [1]. The hypoglycemic effect of the leaf extracts was observed in experimental animals [2], [3]. Clinical tests of the banaba leaf extract to diabetes mellitus were also reported [4], [5].

In our screening test of glucose transporter enhancement activity, banaba was one of the positive samples. Bioassay-guided fractionation of the methanol extract of banaba leaves afforded an active triterpene, corosolic acid (2α-hydroxyursolic acid) [6], which increased the glucose transporter activity *in vitro*, and lowered the blood glucose level in the experimental animals [7]. However, it could not represent the whole activity of the banaba extract. Recently, the objective cell was switched from tumour cells to adipocytes, physiological target cells of insulin. This paper describes the isolation and the identification of more active compounds using the improved methodology.

The aqueous fraction of the acetone extract of banaba leaves, after treatment with ether, ethyl acetate and butanol, was found to possess the ability to increase the 2-deoxyglucose (2-DG) uptake of rat adipocytes. The fraction was loaded onto a Diaion HP-20 column, which was then eluted with a stepwise gradient of H_2O/MeOH. Table **1** shows the results when the aqueous fraction (input) and the eluted fractions were dried, reconstituted to a constant volume, and then assayed for the ability to increase 2-DG uptake. The ability was concentrated

Affiliation: [1] Institute of Pharmaceutical Sciences, Faculty of Medicine, Hiroshima University, Hiroshima, Japan · [2] Faculty of Pharmaceutical Science, Nagasaki University, Nagasaki, Japan

Correspondence: Prof. Kazuo Yamasaki · Institute of Pharmaceutical Sciences · Faculty of Medicine · Hiroshima University · 1-2-3 Kasumi · Minami-ku · Hiroshima 734-8551 · Japan · E-Mail: yamasaki@pharm.hiroshima-u.ac.jp · Fax: +81-82-257-5289

Received: March 29, 2001 · **Accepted:** June 10, 2001

Bibliography: Planta Med 2002; 68: 173–175 · © Georg Thieme Verlag Stuttgart · New York · ISSN 0032-0943

in the 20 and 30% MeOH fractions. From the 30% MeOH fraction, lagerstroemin (**1**) and flosin B (**2**), and from 20% MeOH fraction, reginin A (**3**) were isolated (Fig. **1**) and identified by the comparison of the NMR and optical rotation data with those of the authentic samples [8], [9] previously isolated from the sample plant (*Lagerstroemia flos-reginae* is the synonym of *L. speciosa*).

Lagerstroemin (**1**) increased the rate of 2-DG uptake at a concentration as low as 0.04 mM and the half-maximum stimulation was obtained at around 0.08 mM. At its maximum concentration (0.3 mM), the tannin increased the rate by 0.26 nmol/5 min (Fig. **2**). In the experiment, insulin, a physiological activator of hexose transport, increased the rate by 0.48 nmol/5 min. Flosin B (**2**), an epimer of lagerstroemin, showed a dose-dependent effect similar to lagerstroemin. Reginin A (**3**) also induced a marked increase in the hexose uptake. In this case, however, the effect peaked at 0.04 mM, but it decreased at higher concentrations for an unknown reason.

The mechanism by which the tannins stimulate the hexose uptake is not clear now, but is expected to be similar to that employed by insulin, because wortmannin, an inhibitor of insulin signaling [8], prevented the action of the tannins at the concentrations at which the chemical inhibits insulin actions (data not shown). Thus, we found that three ellagitannins purified from banaba leaves are efficient activators of hexose uptake in rat adipocytes. At their optimum concentrations, the tannins increased the rate of hexose uptake to the level higher than a half of that induced by insulin. The result suggests that the insulin-like action of the ellagitannins or their metaoblites is responsible for the hypoglycemic effect of banaba extract *in vivo* [4], [5].

Materials and Methods

The leaves of *Lagerstroemia speciosa* (Banaba) were collected at the mountain in Zambales in the philippines on March 2000. The voucher specimen (Lag-0037) was deposited in the herbarium of the Institute of Pharmaceutical Sciences, Hiroshima University.

Table **1** Effect of each fraction on 2-DG uptake of rat adipocytes

Fraction	*Concentration* (μg/ml)*	*Increase in2-DG uptake** (nmol/5 min)*
Acetone extract (input)	172	0.49
Eluted fractions		
Water	71	0.24
10% MeOH	9	0.08
20% MeOH	19	0.26
30% MeOH	32	0.33
40% MeOH	14	0.08
50% MeOH	4	−0.01
70% MeOH	1	−0.01
100% MeOH	0.1	−0.01

* The concentration used at assay, which is proportional to the total amount (yield) of each fraction, is presented.

** Basal 2-DG uptake without extract was 0.13 nmol/5 min.

Each result indicates the mean of duplicate determinations.

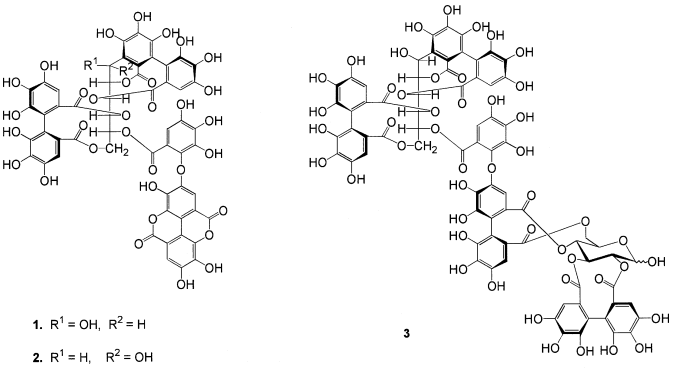

Fig. **1** Chemical structures of lagerstroemin (**1**), flosin B (**2**) and reginin A (**3**).

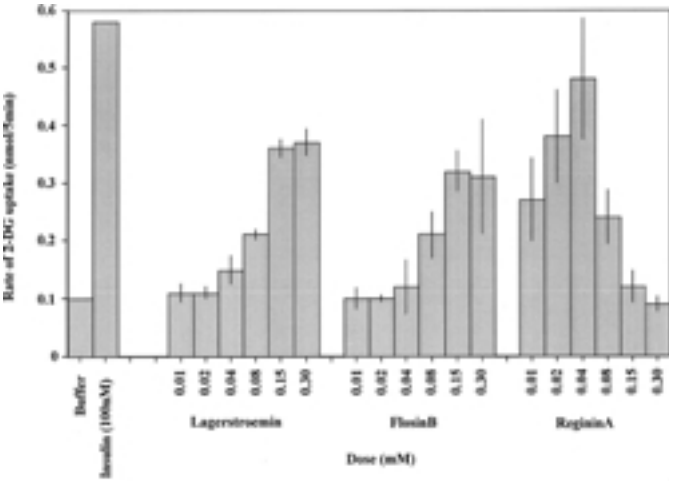

Fig. **2** Effect of tannins on hexose uptake. Rat adipocytes were incubated at 37 °C for 20 min with varied concentrations of lagerstroemin, flosin B, or reginin A duplicate determination. A vertical line indicates the range of duplicate determinations.

Dried leaves of *Lagerstroemia speciosa* (1 kg) were extracted three times with 70% acetone at room temperature. The extract was concentrated under reduced pressure at below 40 °C. The residue (224 g) was suspended in water, and partitioned with ether, ethyl acetate and butanol, successively to afford 1.2 g, 9.2 g, 21 g of the respecitve and 82 g of aqueous fractions. Each fraction was tested for glucose transporter (GT) enhancing activity. The activity was confined to the aqueous fraction, which was subjected to column chromatography on a highly porous copolymer of styrene and divinylbenzene (Diaion HP-20, 6 × 40 cm), with a stepwise gradient elution from water, 10, 20, 30, 40, 50, 70 and 100% MeOH. Each fraction consisted of 2.1 g (5 l elution), 4.4 g (5 l), 7.4 g (5 l), 3.4 g (5 l), 1.0 g (4 l), 0.24 g (2 l) and 0.03 g (2 l), respectively. The activity was concentrated in the 20 and 30% MeOH fractions. A portion of the 30% MeOH fraction (4.0 g) was subjected to MCI-GEL (3 × 27 cm) with elution of aqueous MeOH (0–40%), ODS-AQ column (2.5 × 20 cm) with elution of aqueous MeOH (25%) and preparative HPLC (ODS-A, 2 × 15 cm, 10% CH_3CN-10 mM phosphate buffer, pH = 2.0) to give lagerstroemin (**1**, 27 mg) and flosin B (**2**, 12 mg). From the 20% MeOH fraction (4.0 g) Reginin A (12 mg) was obtained through the same chromatography system (Fig. **1**).

Lagerstreomin (**1**): An off-white amorphous powder, $[\alpha]_D^{20}$: +13.5° (*c* 0.2, acetone), lit. [8] +7.3° (*c* 1.0, acetone); ^{1}H- and ^{13}C-NMR: identical with lit. [8].

Flosin B (**2**): An off-white amorphous powder, $[\alpha]_D^{20}$: +69° (*c* 0.06, acetone), lit. [9] +65° (*c* 1.4, acetone); ^{1}H- and ^{13}C-NMR: identical with lit. [9].

Reginin A (**3**): An off-white amorphous powder, $[\alpha]_D^{20}$: +77° (*c* 0.0026, acetone), lit. [8] +62° (*c* 0.9, acetone); ^{1}H- and ^{13}C-NMR: identical with lit. [8].

Isolation of rat adipocytes and determination of the rate of glucose uptake were performed as described previously [10], [11].

References

[1] Quisumbing E. Medicinal Plants of the Philippines. Katha Publishimg, Quezon City: 1978: 640 – 2

[2] Garcia F. On the hypoglycemic effect of decoction of *Lagerstroemia speciosa* (Banaba) administered orally. Journal of the Philippine Medicinal Associations 1940; 20: 395 – 402

[3] Kakuda T, Sakane I, Takihara T, Ozaki Y, Takeuchi H, Kuroyanagi M. Hypoglycemic effect of extracts from *Lagerstroemia speciosa* L. leaves in genetically diabetic KK-AY mice. Bioscience, Biotechnology and Biochemistry 1996; 60: 204 – 8

[4] Garcia F. Plantisul tablets in the treatment of diabetes mellitus. Journal of the Philippine Medicinal Associations 1956; 31: 216 – 24

[5] Ikeda Y, Chen J-T, Matsuda T. Effectiveness and safety of banabamin tablet containing extract from banaba in patients with mile type 2 diabetes. Japanese Pharmacology & Therapeutics 1999; 27: 829 – 35

[6] Murakami C, Myoga K, Kasai R, Ohtani K, Kurokawa T, Ishibashi S, Dayrit F, Padolina WG, Yamasaki K. Screening of plant constituents for effect on glucose transport activity in Ehrlich ascites tumour cells. Chemical and Pharmaceutical Bulletin 1993; 41: 2129 – 31

[7] Tommasi ND, Simone FD, Cirino G, Cicara C, Pizza C. Hypoglycemic effects of sesquiterpene glycosides and polyhydroxylated triterpenoids of *Eriobotrya japonica*. Planta Medica 1991; 57: 414 – 6

[8] Xu YM, Sakai T, Tanaka T, Nonaka G, Nishioka I. Tannins and related compounds CVI. Preparation of aminoalditol derivatives of hydrolysable tannins having α and β-glucopyranose cores, and its application to the structure elucidation of new tannins, reginin A and B and flosin A isolated from *Lagerstroemia flos-reginae* Retz. Chemical and Pharmaceutical Bulletin 1991; 39: 639 – 46

[9] Xu YM, Tanaka T, Nonaka G, Nishioka I. Tannins and related compounds. CVII. Structure elucidation of three new monomeric and dimeric ellagitannins, flosin B and reginins C and D, isolated from *Lagerstroemia flos-reginae* Retz. Chemical and Pharmaceutical Bulletin 1991; 39: 647 – 50

[10] Okada T, Kawano Y, Sakakibara T, Hazeki O, Ui M. Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes: studies with a selective inhibitor wortmannin. Journal of Biological Chemistry 1994; 269: 3568 – 73

[11] Takasuga S, Katada T, Ui M, Hazeki O. Enhancement by adenosine of insulin-induced activation of phosphoinositide 3-kinase and protein kinase B in rat adipocytes. Journal of Biological Chemistry 1999; 274: 19545 – 50