Antihypertensive Activity of 6-O-Galloyl-D-glucose, a Phenolic Glycoside from Sapium sebiferum

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ANTIHYPERTENSIVE ACTIVITY OF 6-*O*-GALLOYL-D-GLUCOSE, A PHENOLIC GLYCOSIDE FROM *SAPIUM SEBIFERUM*

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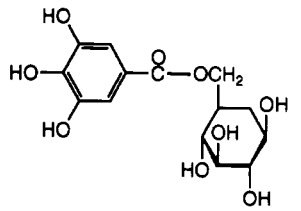
ABSTRACT.—The antihypertensive activity of a phenolic glycoside contained in the leaves of *Sapium sebiferum* was investigated. From intravenous screening using spontaneously hypertensive rats, 6-*O*-galloyl-D-glucose was identified as an active substance. The hypotensive action of this compound appears to be produced by an inhibition of noradrenaline release and/or a direct vasodilatation.

The leaves of *Sapium sebiferum* (L.) Roxb. (Euphorbiaceae) are used as an antiinflammatory drug with diuretic and parasiticidal actions in Chinese traditional medicine (1). In the course of an investigation dealing with hypotensive principles from the plants of Taiwan, a phenolic compound was isolated from the leaves of this plant. In this report, the chemical characteristics and antihypertensive activity of this compound [**1**] are described.



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Polydextran, polystyrene gel and reversed-phase chromatography of the aqueous Me_2CO extract of *S. sebiferum* leaves yielded an active principle [**1**]. The ^{1}H-nmr spectrum of **1** indicated the presence of a galloyl group (δ 7.15, s, 2H) and sugar moiety (δ 3.47–5.18, m, 7H). Its ^{13}C-nmr spectrum exhibited a duplicated signal pattern of sugar carbons and the chemical shifts of anomeric carbon signals at δ 93.3 and 97.7, corresponding to α and β respectively, and are in close agreement with those of glucose. Hydrolysis of **1** with tannase yielded gallic acid and glucose. In addition, the downfield shift of C-6 (δ 64.5) indicated that the galloyl group is located at the C-6 position of the glucose moiety. On the basis of these results, **1** was assigned as 6-*O*-galloyl-D-glucose (2).

Intravenous injection of 6-*O*-galloyl-D-glucose (GDG) into conscious spontaneously hypertensive rats (SHRs) produced a marked hypotensive effect (Figure 1) in a dose-dependent manner from 0.8 mg/kg to 15 mg/kg; a similar degree of blood pressure-lowering effect was also observed in pentobarbitol (30 mg/kg, ip) anesthetized SHRs (data not shown). The effects of GDG reached a plateau within 20 min and were maintained for 35 min or more. The duration of action for GDG was progressively lengthened as the dosage was increased but the effects were completely reversed within 2 h even after a dose of 20 mg/kg. No irreversible inhibition was observed. Similar treatment with prazosin (0.1 mg/kg, iv) to SHRs lowered the blood pressure by 31.4±7.2 mm Hg (n=6). In contrast, GDG at the dose of 5 mg/kg induced a similar hypotensive effect (ΔMAP=29.6±10.4 mm Hg, n=6). There was no statistical difference when comparing the hypotensive action of 0.1 mg/kg of prazosin to that of 5 mg/kg of GDG. Moreover, GDG (1 mg/kg, iv) lowered the blood pressure of

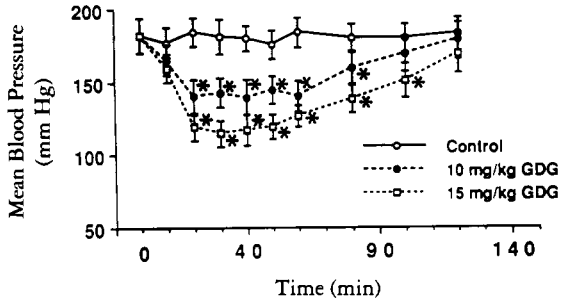

FIGURE 1. The time course of changes in tail arterial mean blood pressure of spontaneously hypertensive rats after bolus injection of 6-*O*-galloyl-D-glucose (GDG). Plotted values represent the mean response of 8 animals and bars represent±S.D. Control rats received an equivalent volume of vehicle. *Significant ($p<0.05$) from the corresponding control value using ANOVA with Dunnett's *t*-test (17).

SHRs to a similar degree as an equal dose of nifedipine (ΔMAP=17.3±7.1 mm Hg vs. 10.8±2.8 mm Hg, n=6). Thus, GDG produced a level of antihypertensive activity in SHRs similar to that seen for prazosin, a blocker of α_1-adrenoceptors (3), and to that for nifedipine, an antagonist of calcium channels (4). Because GDG belongs to the family of ellagitannins that are characterized by their inability to enter the central nervous system (5), participation of central nervous system factors in this phenol-induced antihypertension seems unlikely.

Plasma samples were taken 20 min after the animals received a single bolus intravenous injection of GDG, the time for maximal hypotensive action. Plasma samples from rats treated with only the vehicle were used as controls. Plasma noradrenaline levels in anesthetized SHRs were markedly reduced by treatment with GDG at doses sufficient to produce an antihypertensive action (Table 1). Decrease of plasma noradrenaline seemed to parallel the antihypertensive action seen in GDG-treated animals. In addition, GDG did not interfere with the assay of noradrenaline; addition of GDG at 1 mmol/ml into test tubes containing standard noradrenaline gave a 97.9±3.6% (n=8) detection. Therefore, an inhibitory effect of GDG on the release of noradrenaline, either the rate and/or the amount, can be considered.

In adrenalectomized SHR under anesthesia, mean arterial blood pressure was 172.4 mm Hg, a value which was not statistically different from that in sham-operated SHRs (181.8 mm Hg, Table 1). Intravenous injection of GDG lowered the blood pressure and decreased the plasma noradrenaline in adrenalectomized SHRs in a fashion similar to that for the sham-operated animals (Table 1). No statistical difference was found between the samples from adrenalectomized and sham-operated rats. Such results would seem to exclude the involvement of the adrenal gland, the other source of endogenous catecholamine (6). Thus, peripheral noradrenergic neurons seem responsible for the action of GDG. This is similar to the effects seen in pithed rats directly (7,8). Interference with sympathetic traffic or function may also count, in part, for the absence of tachycardia in GDG-treated animals. Normally, tachycardiac responses would be provoked by a fall in blood pressure and reflex stimulation of sympathetic nerve activity (9,10).

In anesthetized normotensive rats,

TABLE 1. Effect of Intravenous 6-*O*-Galloyl-D-glucose (GDG) on Plasma Noradrenaline Level and Mean Blood Pressure in Spontaneously Hypertensive Rats (SHRs) with Sham Operation (Sham) or Adrenalectomy (Adex).

GDG Dosage (mg/kg)	Plasma Noradrenaline[a] (pmoles/ml)		Mean Blood Pressure[a] (mm Hg)	
	Sham	Adex	Sham	Adex
Control	2.51±0.09	2.18±0.06	181.8±11.6	172.4±10.3
1	2.37±0.07*[b]	2.04±0.09*[b]	168.4±9.4*[b]	161.1±10.2*[b]
5	2.04±0.06**[b]	1.76±0.07**[b]	151.2±10.3**[b]	143.8±9.8**[b]
10	1.62±0.08**[b]	1.49±0.08**[b]	140.9±11.7**[b]	132.4±10.7**[b]
15	1.28±0.05**[b]	1.12±0.07**[b]	118.7±8.9**[b]	110.4±7.2**[b]

[a]Tabular values represent the mean±S.D. for 8 animals.

[b]Significance, $*p<0.05$ and $**p<0.01$, as compared with respective control through Dunnett's *t*-test (17).

bolus injection of methoxamine (0.1 μg/kg) raised mean arterial blood pressure, while an injection of 1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)-phenyl]-3-pyridine carboxylic acid ester (Bay-K8644, 10 μg/kg) also elevated it markedly (Table 2). The vasoconstrictive response to methoxamine in rats was significantly attenuated by GDG after a 20-min intravenous pretreatment. A dose-related reduction in vasopressive response was also observed in Bay-K8644-stimulated rats (Table 2). No statistical difference ($p>0.05$) could be found between the effects of GDG-induced inhibition against the vasoconstrictive responses to methoxamine and Bay-K8644. Bay-K8644 and methoxamine induce vasoconstriction through different action mechanisms, either the opening of calcium L-channels (11) or the activation of α-adrenoceptors (12) to mobilize calcium ions in vascular vessels. Therefore, a direct effect of GDG on vascular activity seems responsible for the production of this inhibition.

In conclusion, the data suggest that 6-*O*-galloyl-D-glucose (GDG) possesses the ability to lower the mean blood pressures of SHR through the blockade of noradrenaline release and/or direct vasorelaxation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with a Jasco DIP-140 digital polarimeter. Ir spectra were obtained with a Jasco A-100 spectrometer. The ^{1}H- and ^{13}C-nmr spectra were taken with Bruker AM-300 and JEOL EX-

TABLE 2. Effect of Intravenous Pretreatment with 6-*O*-Galloyl-D-glucose (GDG) on the Vasopressive Responses of Methoxamine and Bay-K8644 in Rats.

GDG Dosage (mg/kg)	Δ MAP (mm Hg)[a]	
	Methoxamine (0.1 μg/kg)	Bay-K 8644 (10 μg/kg)
Control	47.2±6.9	43.7±7.5
5	36.3±5.8*[b]	34.1±4.6*[b]
10	29.8±4.7**[b]	23.6±5.8**[b]
15	17.1±6.3**[b]	14.3±6.2**[b]

[a]Tabular values represent the mean±S.D. for 6 animals.

[b]Significance, $*p<0.05$ and $**p<0.01$, from the respective vehicle-treated control via Dunnett's *t*-test (17).

90 spectrometers, using TMS as an internal standard: chemical shifts are given in δ (ppm). Cc was carried out with Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical), Diaion HP-20AG, MCI-gel CHP-20P (75–150 μm, Mitsubishi Chemical Industries), Fuji-gel ODS (43–65 μm, Fuji Gel Hanbai) and Bondapak C_{18}/Porasil B (37–75 μm, Waters). Tlc was carried out on precoated Kieselgel 60 F_{254} plates (0.2 mm thick, Merck) with the solvent systems C_6H_6-HCOOEt-HCOOH (1:7:1 and 3:6:1) or on precoated cellulose F_{254} plates (0.1 mm thick, Merck) using the solvent system *n*-BuOH-pyridine-H_2O (6:4:3); tlc detection was by uv illumination and by spraying with 5% EtOH $FeCl_3$/EtOH or aniline-hydrogen phthalate reagents.

PLANT MATERIAL.—Leaves of *Sapium sebiferum* (L.) Roxb. (Euphorbiaceae) were collected from Taipei Mountain in Taiwan in May 1991, under the direction of Mr. M.T. Kao, Department of Botany, National Taiwan University. Voucher specimens are deposited in the Herbarium of the School of Pharmacy, Taipei Medical College, Taipei, Taiwan.

EXTRACTION AND ISOLATION.—Fresh *Sapium sebiferum* leaves (6.0 kg) were macerated in 80% aqueous Me_2CO overnight at room temperature. The extract was then concentrated *in vacuo* at 40° to remove the solvent, the residual aqueous solution was filtered, and the filtrate was chromatographed over a Sephadex LH-20 column with H_2O/MeOH as eluent. Based on bioactivity examination, the first 20% MeOH eluate was discarded, and the active 30–50% MeOH eluate (fraction 2) was collected. The 60–70% MeOH eluate did not contain activity. Fraction 2 was processed using a Diaion HP-20 AG column (H_2O-MeOH) and the subsequent 30–40% MeOH eluate was collected, then subjected to a MCI CHP-20P column (H_2O/MeOH), wherein the fraction of 20–30% MeOH eluate was purified using a Bondapak C_{18} column with 20–25% MeOH in H_2O. 6-*O*-Galloyl-D-glucose (220 mg) was obtained after the eluate was concentrated under reduced pressure.

6-*O*-GALLOYL-β-D-GLUCOSE.—Colorless needles (H_2O); $[\alpha]^{25}D$ +25.3° (*c*=0.5, MeOH); mp 137–139°; ir (KBr) ν max 3000–3600 (OH), 1700 (C=O), 1610 (C=C) cm^{-1}; ^1H nmr (Me_2CO-d_6+D_2O) δ 3.47–5.18 (7H, m, sugar-H), 7.15 (2H, s, galloyl-H); ^{13}C nmr (Me_2CO-d_6+D_2O) δ 64.5 (C-6α, β), 70.3 (C-4α), 71.0 (C-4β), 71.2 (C-2α), 73.1 (C-5β), 74.2 (C-3α), 74.7 (C-2β), 75.6 (C-5β), 77.3 (C-3β), 93.3 (C-1α), 97.7 (C-1β), 109.7 (galloyl C-2, C-6), 121.2 (galloyl C-1), 138.8 (galloyl C-4), 145.9 (galloyl C-3, C-5), 167.2 (-COO-). Hydrolysis of 6-*O*-galloyl-β-D-glucose with tannase at 37° furnished gallic acid and D-glucose, which were identified by tlc with authentic samples.

ANIMALS.—Spontaneously hypertensive rats (SHR) were obtained from the animal center of National Cheng Kung University Medical Center. They weighed 250–280 g and were kept in a room at constant temperature (26±1°) with a 12 h light cycle and with free access to food and H_2O.

DETECTION OF BLOOD PRESSURE.—Systemic blood pressure of awake animals was measured as previously described (13) using an indirect tail-cuff method. A photoelectric sensor was used to detect pressure pulses through a cuff pump (IITC, Mod. 20) and pulse amplifier (IITC, Mod. 59). Recording on the computer program was carried out at room temperature (28±1°) to avoid a stressful pre-warming procedure. The accuracy and reliability of this method have been described previously (14).

ANTIHYPERTENSIVE ACTIVITY.—SHR animals with a systemic blood pressure higher than 180 mm Hg were employed for the screening of antihypertensive activity. Test compounds were dissolved in Locke-Ringer solution. After intravenous injection of each substance into the cannulated femoral vein within 20 sec at the desired dose, systemic blood pressure was measured at 10-min intervals. Lowering of tail arterial blood pressure to a significant level ($p<0.05$) relative to control blood pressure before administration of the test compound, was used to indicate activity. Only a substance which produced a hypotensive effect lasting 30 min or longer was considered to possess antihypertensive activity.

ASSAY OF PLASMA NORADRENALINE.—Blood samples (2 ml), collected from the optic sinus of ether-anesthetized rats, were centrifuged at 5,000×*g* for 10 min at 4° and aliquots (200 μl) of plasma were removed for assay. Plasma samples added with 20 ng of dihydroxybenzylamine (DHBA), the internal standard, were adsorbed onto activated alumina by continuous shaking for 30 min. The alumina was then washed three times with 1 ml of distilled H_2O. The catechols were eluted by 0.1 M perchloric acid with a 10-min shaking period. Quantitation of noradrenaline in the clear supernatant was performed (15) using hplc with an electrochemical detector (BAS200). All values, after correction of recovery (83–87%), were expressed as pmoles per ml.

ADRENALECTOMIZED RATS.—Bilateral adrenalectomy was carried out following previously published method (16) using the dorsal approach under Et_2O-anesthesia. Sham-operated animals served as control. The animals were allowed to recover from the surgery for 24 h before being used for experiments.

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