Antihypertensive Activity of Corilagin and Chebulinic Acid, Tannins from Lumnitzera, racemosa

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ANTIHYPERTENSIVE ACTIVITY OF CORILAGIN AND CHEBULINIC ACID, TANNINS FROM *LUMNITZERA RACEMOSA*

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ABSTRACT.—The antihypertensive activity of eleven hydrolyzable tannins contained in the leaves of *Lumnitzera racemosa* (Combretaceae) was investigated. From the screening in spontaneously hypertensive rats, corilagin, castalagin, and chebulinic acid were identified as the major active substances. This action of corilagin and chebulinic acid has not been mentioned before.

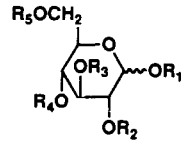
The plants of *Lumnitzera racemosa* Willd. (Combretaceae) are large glabrous shrubs, growing in salt marshes along with mangroves in Taiwan. There is very little knowledge of its phytochemistry (1). According to the folk medicine, the fruits of this plant are curative in skin disorders (2).

To develop new treatments for hypertension, a series of experiments were conducted and a preliminary screening of *L. racemosa* aqueous Me_2CO extract confirmed its antihypertensive activity. Eleven hydrolyzable tannins were identified and were injected into spontaneously hypertensive rats to evaluate the blood pressure lowering activity. This paper describes the isolation, structural characterization, and biological activity of these compounds.

EXPERIMENTAL

PLANT MATERIALS.—Leaves of *L. racemosa* were collected from Kaohsiung Bay in Taiwan during August of 1991. The voucher specimens have been deposited in the herbarium of the Graduate Institute of Pharmaceutical Sciences in Taipei Medical College.

ISOLATION PROCEDURES.—The air-dried leaves of *L. racemosa* (800 g) were soaked in 60% aqueous Me_2CO at room temperature overnight. After three extractions, the combined extract was concentrated under reduced pressure followed by filtration to remove brown precipitates. The filtrate was concentrated and subjected to Sephadex LH-20 cc with H_2O containing increasing amounts of MeOH and finally with H_2O-Me_2CO (1:1) to give three fractions: Fr. I (0.54 g), Fr. II (3.2 g), and Fr. III (2.4 g). Fr. I was then chromatographed over Fuji-gel ODS G3, Bondapack C18/Porasil B (H_2O/MeOH), and Sephadex LH-20 (EtOH) to yield 44 mg of 2,3-(*S*)-HHDP-D-glucose [**9**]. Repeated chromatography of Fr. II on MCl-gel CHP 20P, Fuji-gel ODS G3, Bondapack C18/Porasil B (H_2O/MeOH) and Sephadex LH-20 (H_2O/MeOH, EtOH) yielded 28 mg of 2,3-di-*O*-galloyl-D-glucopyranose [**5**], 10 mg of 1,2,3,6-tetra-*O*-gal-



G = galloyl
HHDP = hexahydroxydiphenoyl
CHEB = chebuloyl
NECH = neochebuloyl
GALA = gallagyl

	R_1	R_2	R_4	R_3	R_5
1	G	H	H	HHDP	
2	G	CHEB		HHDP	
3	G	CHEB		G	G
4	G	NECH		G	G
5	H	G	H	G	H
6	G	G	H	G	G
7	H	G	G	G	G
8	G	G	G	G	G

	R_1	R_2	R_3	R_4	R_5
9	H	HHDP		H	H
10	H	HHDP		GALA	

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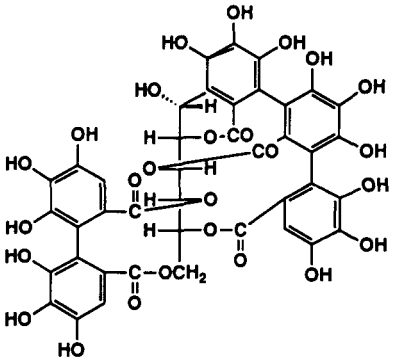
loyl-D-glucopyranose [**6**], 322 mg of corilagin [**1**], and 10 mg of chebulagic acid [**2**]. From Fr. III, similar chromatography yielded 22 mg of 2,3,4,6-tetra-*O*-galloyl-D-glucopyranose [**7**], 9 mg of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose [**8**], 40 mg of castalagin [**11**], 108 mg of punicalagin [**10**], 11 mg of neochebulinic acid [**4**], and 110 mg of chebulinic acid [**3**].



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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°) under a light cycle of 12 h, with free access to food and H_2O.

DETECTION OF BLOOD PRESSURE.—Systemic blood pressure of awake animals was measured by an indirect tail-cuff method according to our previous procedures (3). A photoelectric sensor was used to detect pressure pulses through a cuff pump (IITC, Model 20) and pulse amplifier (IITC, Model 59). Recording on the computer program was carried out at ambient temperature (28 ± 1°) to avoid the stressful prewarming procedure. The accuracy and reliability of this method have been described previously (4).

ANTIHYPERTENSIVE ACTIVITY.—Animals with a systemic blood pressure higher than 180 mmHg were employed for the screening of antihypertensive activity. Tannins for testing were dissolved in the Locke Ringer solution. After iv injection of each substance at the desired concentration, systemic blood pressure was measured at 10-min intervals. Lowering of tail arterial blood pressure at a level of 5% or more, compared to control before administration of testing compound, was considered significant ($p<0.05$). Only a substance that produced a hypotensive effect lasting 30 min or longer was considered to possess antihypertensive activity.

RESULTS AND DISCUSSION

The aqueous Me_2CO extract of dried leaves of *L. racemosa* was subjected to Sephadex LH-20 cc separation through a solvent system of H_2O/MeOH/Me_2CO to give three fractions. After repeated chromatography over polydextran, polystyrene, and various reversed-phase gels, eleven compounds were obtained; all of them were hydrolyzable tannins. Chemical structure of these compounds was identified as 2,3-di-*O*-galloyl-D-glucopyranose [**5**] (5), 1,2,3,6-tetra-*O*-galloyl-β-D-glucopyranose [**6**] (6), 2,3,4,6-tetra-*O*-galloyl-D-glucopyranose [**7**] (7), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose [**8**] (8), 2,3-(*S*)-HHDP-D-glucose [**9**] (9), corilagin [**1**] (9), chebulagic acid [**2**] (10), punicalagin [**10**] (1), castalagin [**11**] (12), neochebulinic acid [**4**] (13), and chebulinic acid [**3**] (14), by comparisons of their physical and spectral data with those of authentic samples or literature.

We screened the antihypertensive activity of these compounds using spontaneously hypertensive rats (SHRs). Bolus injection of the testing substance into animals was employed to rule out the pharmacokinetic factors of absorption. As shown in Figure 1, injection of the same volume (0.1 ml/kg) of vehicle did not modify the mean blood pressure of conscious SHRs. Corilagin [**1**], chebulinic acid [**3**], and castalagin [**11**] lowered the blood pressures of SHRs at different onset times (Figure 1). The antihypertensive action of these compounds was produced in a dose-dependent manner, as illustrated in Table 1. The maximal changes in blood pressure (mmHg) of SHRs show that chebulinic acid [**3**] works more effectively than corilagin [**1**], while the activity of castalagin [**11**], one of the antihypertensive principles we have observed in the leaves of *Melastoma candidum* (submitted for publication), is similar to corilagin [**1**] (Table 1). Moreover, these compounds also produced a hypotensive effect in

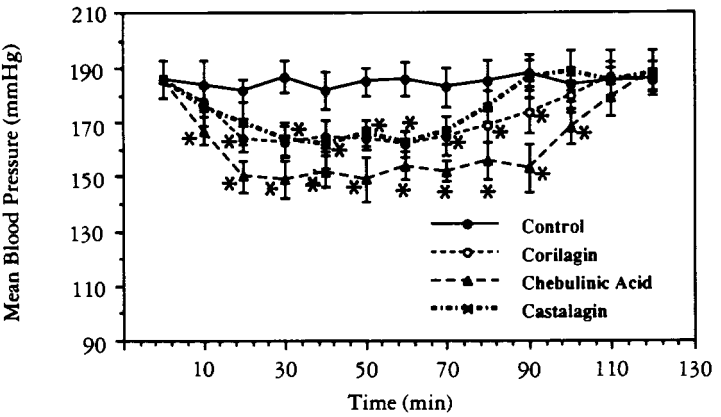

FIGURE 1. The time course of changes in tail arterial mean blood pressure of spontaneously hypertensive rats after bolus injection of tannins at 5 mg/kg. Values are the means from 8 animals, and bars represent SEM. The control is obtained from rats receiving same volume of vehicle. $^*P<0.05$ from the value of control at same stage using ANOVA with Dunnett's test.

normotensive (WKY) rats. At the maximal dose obtained in SHRs, chebulinic acid [**3**] lowered the pressure from preinjection about 44.6 ± 5.2 mmHg ($n = 6$), while corilagin [**1**] reduced it by 36.7 ± 6.1 mmHg ($n = 6$) and castalagin [**11**] decreased it by 35.4 ± 7.2 mmHg ($n = 6$). Similar injection of vehicle at the same volume failed to modify the mean blood pressure of conscious normotensive rats ($n = 6$). There is no statistical difference ($P > 0.05$) in the actions of these compounds between normotensive rats and SHRs either in the onset time or the effective dose (ED_{50}): 3.4 ± 1.1 mg/kg for chebulinic acid [**3**], 6.5 ± 0.9 mg/kg for corilagin [**1**], and 6.4 ± 1.3 mg/kg for castalagin [**11**] in normotensive rats ($n = 6$). This suggested that these active tannins possess the ability to lower blood pressures of both SHRs and normotensive rats.

In the screening with SHRs at 5 doses, as shown in Table 1, compounds

TABLE 1. Effect of Hydrolyzable Tannins on the Arterial Blood Pressure of Spontaneously Hypertensive Rats.

Tannin	Testing Dose[a] (mg/kg) (ED_{50} at mg/kg)	Maximal effect[b] (mmHg)
Corilagin [**1**]	0.5–20 (6.7 ± 1.4)	-52.6 ± 7.1^c ($n = 8$)
Chebulagic acid [**2**]	1–50	-6.5 ± 5.3 ($n = 8$)
Chebulinic acid [**3**]	0.5–10 (3.2 ± 0.9)	-76.9 ± 6.2^c ($n = 8$)
Neochebulinic acid [**4**]	1–50	-8.9 ± 7.3 ($n = 8$)
2,3-Di-*O*-galloyl-D-glucopyranose [**5**]	1–50	4.5 ± 3.7 ($n = 6$)
1,2,3,6-Tetra-*O*-galloyl-D-glucopyranose [**6**]	1–50	2.3 ± 1.9 ($n = 6$)
2,3,4,6-Tetra-*O*-galloyl-D-glucopyranose [**7**]	1–50	-5.2 ± 4.8 ($n = 6$)
1,2,3,4,6-Penta-*O*-galloyl-β-D-Glucopyranose [**8**]	1–50	-7.1 ± 6.4 ($n = 6$)
2,3-(*S*)-HHDP-D-glucose [**9**]	1–50	-5.5 ± 4.3 ($n = 6$)
Punicalagin [**10**]	0.5–15 (5.4 ± 1.1)	63.4 ± 7.7^c ($n = 8$)
Castalagin [**11**]	0.5–20 (6.3 ± 1.2)	-49.6 ± 5.4^c ($n = 8$)

[a]Testing dose means the dose range of testing tannins injected at 5 concentrations (mg/kg) into rats (n = number) to estimate the dose-response curve for calculating the effective dose (ED_{50}).

[b]Maximal effect indicates the peak changes of blood pressure (mean ± SEM) in rats receiving the bolus injection of tannins at maximal dose from that obtained at preinjection.

[c]$P < 0.01$.

5–9 were inactive in the production of hypotensive activity; this indicates that the galloyl glucose moiety may not be essential for this bioactivity. It is of special interest to observe that chebulagic acid [**2**] and neochebulinic acid [**4**], derivatives of **1** and **3**, respectively, lacked the activity of the parent compound. In contrast, punicalagin [**10**] elevated the blood pressure instead of causing a hypotensive effect. Similar hypertensive action can be obtained in normotensive rats that were treated with punicalagin [**10**]. The presence in *L. racemosa* of analogues with opposite effects seems reasonable, since the crude extract did not possess a marked hypotensive effect. However, participation of the other principles contained in this plant cannot be totally ruled out.

In conclusion, eleven hydrolyzable tannins contained in the leaves of *L. racemosa* were isolated. Three compounds were shown to have hypotensive effect although one of them was hypertensive in rats.

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LITERATURE CITED

1. H.L. Li, "Flora of Taiwan (III)," Epoch Publishing Co., Taipei, Taiwan, 1977, p. 875.
2. W.S. Kan, "Manual of Medicinal Plants in Taiwan," National Research Institute for Chinese Medicine, Taipei, Taiwan, 1970, Vol. 3, p. 601.
3. J.T. Cheng, S.S. Chang, and I.S. Chen, *Arch. Int. Pharmacodyn.*, **306**, 65 (1990).
4. R.D. Bunag and J. Butterfield, *Hypertension*, **4**, 898 (1982).
5. M.A.M. Nawwar, A.M.A. Souleman, J. Buddruns, H. Bauer, and M. Linscheid, *Tetrahedron Lett.*, **25**, 49 (1984).
6. M. Nishizawa, T. Yamagishi, G. Nonaka, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, 961 (1982).
7. R. Armitage, G.S. Bayliss, J.W. Gramshaw, E. Haslam, R.D. Haworth, K. Jones, H.T. Rogers, and T. Searle, *J. Chem. Soc. C*, 1842 (1961).
8. M. Nishizawa, T. Yamagishi, G. Nonaka, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, 963 (1982).
9. M. Seikel and W.E. Hills, *Phytochemistry*, **9**, 1115 (1970).
10. O.T. Schmidt and W. Nieswandt, *Liebigs Ann. Chem.*, **568**, 165 (1950).
11. T. Tanaka, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **34**, 650 (1986).
12. G. Nonaka, T. Sakai, T. Tanaka, K. Mihashi, and I. Nishioka, *Chem. Pharm. Bull.*, **38**, 2151 (1990).

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