Communications to the Editor

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THE Ca^{2+} ANTAGONIST ACTIVITY OF LIGNANS

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A number of lignans, including those isolated from Arctium lappa (Compositae), were subjected to Ca^{2+} antagonist assay. The most potent activity occurred in trachelogenin, the calculated Ic50 and pA_2 of which were $1.1X10^{-6}$ M and 6.60 respectively. Trachelogenin had a potent and long-lasting antihypertensive effect on spontaneously hypertensive rats.

KEYWORDS —— Ca^{2+} antagonist; Arctium lappa; lignan; trachelogenin; arctigenin; antihypertensive effect

The term Ca^{2+} antagonist was first introduced by Fleckenstein,[1] and defined as a group of drugs that inhibit slow transsarcolemmal inward Ca^{2+} current without affecting the Na^+-dependent excitatory process in cardiac and smooth muscle contraction. For the last decade Ca^{2+} antagonists have been used as therapeutic agents, particularly to treat coronary heart diseases and hypertension, and as chemical probes in basic pharmacological research. An increasing demand for Ca^{2+} antagonists as clinical medicines has prompted us to introduce a Ca^{2+} antagonist assay system into our long-term research program projected to isolate biologically active principles from traditional herbal medicines.[2]

Hot aqueous extracts of more than 150 Chinese herbal medicines prepared as described before[2] were tested for Ca^{2+} antagonist activity using the taenia coli of guinea pigs. The preliminary results demonstrate that potent activity exists in hot aqueous extracts of the fruit of Arctium lappa (Compositae, Japanese name: Goboushi). Most of the Ca^{2+} antagonist activity occurred in the methanol fraction, where a number of Arctium lignans were identified on TLC by comparison with authentic samples.[3] In this communication we report the structure-activity relationship of Arctium lignans and related compounds, with respect to Ca^{2+} antagonist activity, and their antihypertensive effects on spontaneously hypertensive rats.

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MATERIALS AND METHODS

Assay for Ca^{2+} Antagonistic Activity The taenia coli of male guinea pigs weighing 300g to 650g were isolated and mounted in an organ bath filled with Krebs solution. K^+ contracture was induced by 40 mM KCl, then samples of the test compounds were administered cumulatively. In evaluating the samples' inhibition of the K^+ contracture, the relaxation of the taenia coli induced by adding verapamil at the concentration of 10^{-5} M was put at 100%. The pA_2 values were obtained by the Magnus method using spiral strips of aortae of male Wistar rat, weighing about 300 g, as follows. The strips were successively equilibrated in modified Krebs-Henseleit solution for 1 h, in Ca^{2+}-free solution containing 3 mM EGTA for 15 min, and in Ca^{2+}-free solution with high K^+ concentration. Adding Ca^{2+} cumulatively to the system caused step-by-step contraction. The pA_2 values were calculated according to Schild's method.[4]

Assay for Antihypertensive Activity of Lignans Male spontaneously hypertensive rats (SHR-NCrj, 16-week-old, obtained from Nippon Charles River Co.) weighing about 330g were anesthetized with pentobarbital sodium (50 mg/kg i.p.) and urethane (1.75 g/kg s.c.). Then doses of each test compound (10 mg/kg) were administered intravenously to respective groups of the rats. Blood pressure was measured directly by carotid artery cannulation.

Test Samples Some of the test samples were offered by Mr. S. Yamanouchi, Dr. A. Ichihara and Dr. K. Tomioka. Each sample was administered in water solution solubilized by the addition of 5 parts of polyvinylpyrrolidone (PVP, MW40,000). The presence of PVP did not affect this assay system.

RESULTS AND DISCUSSION

The lignans can be biogenetically characterized as oxidatively coupled products of two or more phenyl propanoids, and distinguished by their diverse structural variation and complexity. The structural types of lignans tested in this report include bis-tetrahydrofurans (I, II), butanolides (III, VI, VII), an aryltetrahydronaphthalene (IV), a tetrahydrofuran (V) and steganes (VIII). The assay results summarized in Table I demonstrate that of 33 lignans tested, 17 compounds had significant inhibitory potency with Ic50 below 10^{-5}M. The order of inhibitory potencies is: butanolides > bis-tetrahydrofurans = steganes. The tetrahydrofuran and aryltetrahydronaphthalene have no significant acitivity. A wide range of substituents on the aromatic rings (hydroxy, methoxy or methylenedioxy group) did not modify the measured inhibitory potencies, except glucosides which caused large losses of activity in all types. The main feature of chemical structures affecting the activities was the fixation of side benzyl groups by ring formation which dramatically reduced the activities (compare IIIa-c, f-g with VII). The introduction of oxygen functions at the benzylic position, though only a minor modification, also caused a large loss of inhibitory potency (compare VIb-d with VIa). Substantial activities in steganes, though its side benzyls are fixed as in VII, may be ascribed to the flexibility of the 8-membered ring as compared to the rigid ring of VII.

The most potent activity occurred in trachelogenin (IIIf), a typical butanolide, requiring a concentration of 1.1×10^{-6} M for 50% inhibition of the K^+

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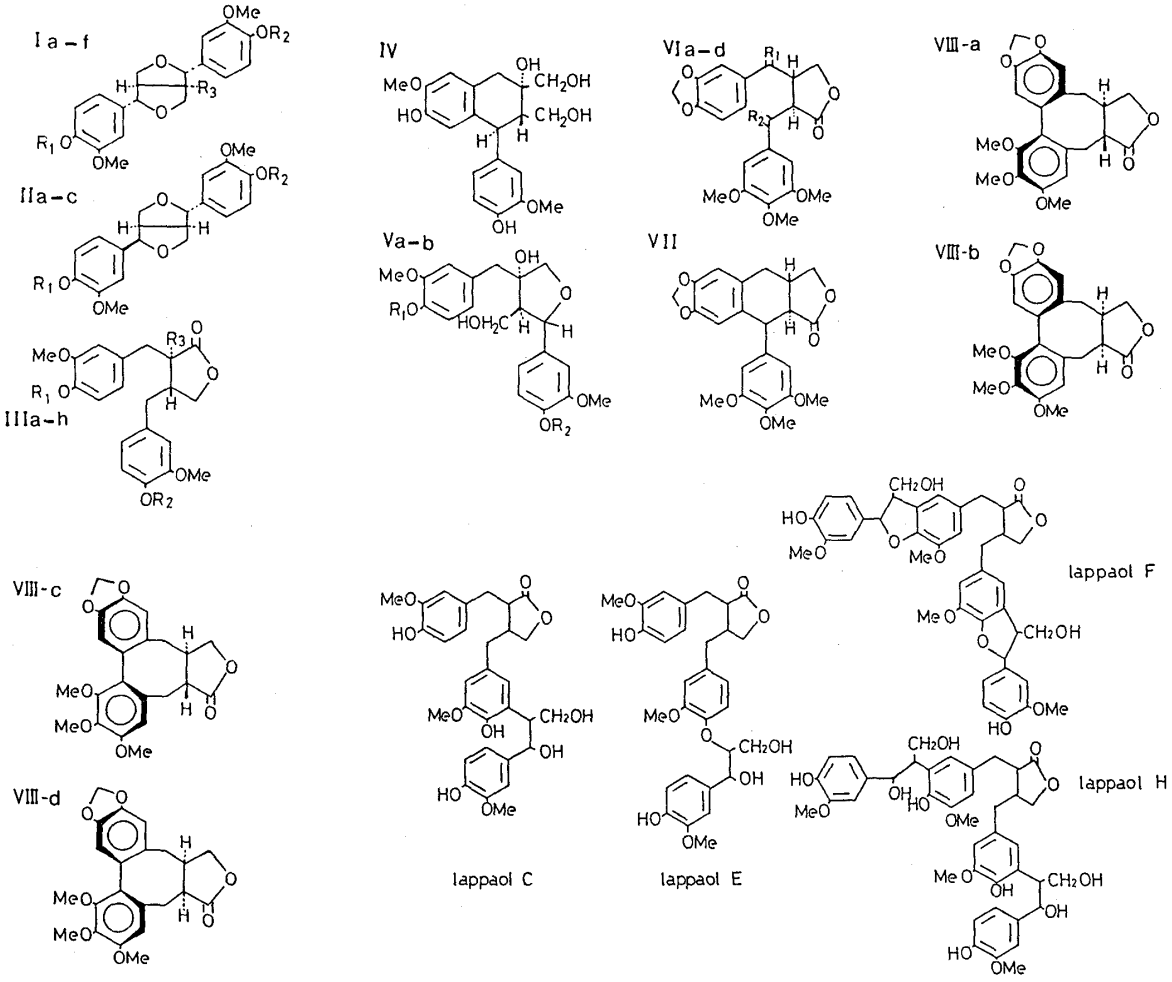

Table I. Inhibitory Activity of Lignans on K^+ Contracture

Compound No.	Substituents R_1	R_2	R_3	Ic50 ($\times 10^{-5}$M)	Compound No.	Substituents R_1	R_2	R_3	Ic50 ($\times 10^{-5}$M)
Ia	H	H	H	4.8	IV				-
Ib	Me	Me	H	4.4	Va	H	H		-
Ic	Glu	H	H	-	Vb	Me	Me		-
Id	Glu	Glu	H	-	VIa	H_2	H_2		1.1
Ie	Me	Me	OAC	3.7	VIb	H,OH	H_2		7.0
If	Me	Me	OH	-	VIc	O	H_2		9.6
IIa	H	H		3.8	VId	H_2	O		-
IIb	Me	H		5.2	VII				-
IIc	Me	Glu		-	VIIIa				3.9
IIIa	H	H	H	1.4	VIIIb				0.78
IIIb	H	Me	H	0.88	VIIIc				0.52
IIIc	Me	Me	H	1.6	VIIId				-
IIId	Glu	H	H	-	IX	lappaol C			-
IIIe	Glu	Me	H	-	X	lappaol E			0.66
IIIf	H	Me	OH	0.11	XI	lappaol F			-
IIIg	Me	Me	OH	0.80	XII	lappaol H			-
IIIh	Glu	Me	OH	-					

(-) indicates that Ic50 was above 10^{-4}M.

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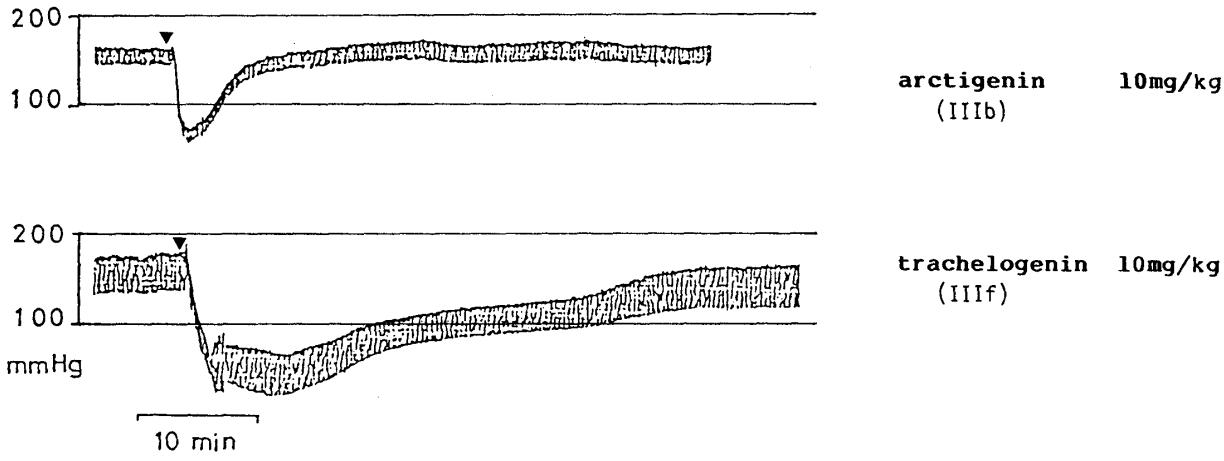

Fig. 1. Antihypertensive Effect of Lignans on Spontaneously Hypertensive Rats

contracture, and its calculated pA_2 value was 6.60, which is one-sixth that of verapamil, a Ca^{2+} antagonist of wide clinical use.[5)]

Some lignans with a distinct inhibitory activity on K^+ contracture were also tested for their antihypertensive effects using spontaneously hypertensive rats. The results shown in Fig. 1 demonstrate that trachelogenin is highly potent and has a relatively long-lasting antihypertensive effect. It has been reported that in spontaneously hypertensive rats the ability of the vascular smooth muscle membrane to retain Ca^{2+} and the ATP-dependent Ca^{2+} uptake of the sarcoplasmic reticulum are reduced.[6)] Ca^{2+} antagonists have been known to inhibit influx responsible for excitation and contraction in the electromechanical coupling, thus blocking contraction of myocardial and vascular muscles and inducing their relaxation.[6)] These functions by Ca^{2+} antagonists are expected to contribute to the lowering of elevated blood pressure. Similarly, it appears that the antihypertensive effect of trachelogenin is attributed to a Ca^{2+} antagonism activity in this compound.

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