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ANTIHEPATOTOXIC PRINCIPLES OF *ATRACTYLODES* RHIZOMES[1]

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ABSTRACT.—Extracts of various samples of the crude drug prepared from *Atractylodes* rhizomes exhibited antihepatotoxic activity by *in vitro* assay methods using carbon tetrachloride- and galactosamine-induced cytotoxicity in primary cultured rat hepatocytes. The main sesquiterpenoid components were subjected to screening by these methods to reveal that atractylon, β-eudesmol, and hinesol exerted significant liver-protective effects. Some analogs were also assayed.

The crude drug "jutsu" is prepared from the rhizomes of *Atractylodes* plants (Compositae) and is empirically classified into two groups: Atractylodes Rhizoma from *A. macrocephala* Koidzumi (*A. ovata* De Candolle) and *A. japonica* Koidzumi, and Atractylodes Lanceae Rhizoma from *A. lancea* De Candolle and its varieties. In oriental medicine, the drug has been prescribed clinically for diuretic and analgesic purposes as well as for stomachic disorders, chiefly to improve water metabolism (1). This paper deals with antihepatotoxic principles of this crude drug.

RESULTS AND DISCUSSION

During the course of our survey for antihepatotoxic principles in plants, we carried out the screening of various samples of this crude drug (2) by means of the *in vitro* assay method that uses carbon tetrachloride (CCl_4)-induced cytotoxicity in primary cultured rat hepatocytes (3); we found that some samples of both Atractylodes Rhizoma and Atractylodes Lanceae Rhizoma exerted significant antihepatotoxic actions, although the activity varied remarkably with lot (table 1). For the samples that exhibited antihepatotoxic activity, assessment of liver-protective effects was also performed by the *in vitro* assay method using D-galactosamine (GalN)-produced cytotoxicity in primary cultured rat hepatocytes (4) to reveal that the samples also gave similar positive responses (table 1).

Because the main components of Atractylodes Rhizoma and Atractylodes Lanceae Rhizoma are known to be atractylon (5) and β-eudesmol and hinesol (6), respectively, these sesquiterpenoids were subjected to evaluation for antihepatotoxic activity by both *in vitro* assay methods. As a result, we found that all the sesquiterpenoids elicited significant preventive effects against CCl_4- and GalN-induced lesions in primary cultured rat hepatocytes (table 2).

In Atractylodes Rhizoma, there are other known constituents: atractylenolide I, II, and III. Although these three sesquiterpenoid lactones were isolated as antiinflammatory principles (7), the latter two had previously been obtained as auto-oxidation products of atractylon (5). Assay of these sesquiterpenoid lactones for antihepatotoxic activity did not afford positive results compatible with those of atractylon (table 2).

In these two assay methods, it is likely that substances that inhibit GPT activity would give positive results. Therefore, direct effects of atractylon, β-eudesmol, and hinesol on the enzyme activity were examined to reveal that the GPT values of the atractylon-, β-eudesmol-, and hinesol-treated groups were 106, 106, and 105%, as compared with that of the control group, indicating that these sesquiterpenoids show no direct effect on the transaminase activity.

[1]Part 9 in liver-protective drugs. This paper also forms Part 54 in the series on the validity of the oriential medicines.

TABLE 1. Effect of *Atractylodes* rhizomes on carbon tetrachloride- and galactosamine-induced cytotoxicity in primary cultured rat hepatocytes (n=3 dishes).

Original plant	Habitat	MeOH ext. yield (%)	Dose (mg/ml)	GPT (%)	
				CCl_4	GalN
Control	—	—	—	100±8	100±2
A. japonica	Japan	5.6	1	89±2	
A. japonica	Japan	14.0	1	74±3	
A. japonica	Japan	19.6	1	75±4	
A. japonica	Japan	25.0	1	61±2[a]	91±2
A. japonica	Japan	33.9	1	74±4	
A. japonica	Japan	27.0	1	73±6	
A. japonica	S. Korea	11.4	1	73±2	
A. japonica	S. Korea	35.0	1	93±3	
A. japonica	S. Korea	27.8	1	48±2[a]	66±3[b]
A. japonica	S. Korea	23.1	1	65±1	
A. japonica	N. Korea	24.7	1	53±5[a]	
A. ovata	China	17.6	1	74±4	72±1[b]
A. ovata	China	19.8	1	80±5	
A. lancea	China	41.7	1	71±3	
A. lancea	China	29.3	1	88±4	
A. lancea	China	31.6	1	33±2[a]	68±2[b]
A. lancea var. *simplicifolia*	Korea	32.0	1	78±2	
A. lancea var. *simplicifolia*	N. Korea	27.1	1	62±3	69±3[a]
A. lancea var. *chinensis*	China	31.0	1	40±3[a]	69±1[b]
A. lancea var. *chinensis*	China	16.2	1	59±4	
A. lancea var. *chinensis*	China	21.2	1	63±2	
A. lancea var. *chinensis* × *A. japonica* ?	China	29.6	1	93±6	

[a]Significantly different from the control, $p < 0.01$.
[b]Significantly different from the control, $p < 0.001$.

It was also probable at this stage that atractylon, β-eudesmol, and hinesol showed their antihepatotoxic effects after metabolic degradation to yield active metabolites *in vivo*. From the above observation that atractylenolide II and III, auto-oxidation products of atractylon and possible *in vivo* metabolites of atractylon, disclosed no (or only weak) preventive actions against experimental liver damage, it was considered to be unlikely that atractylon displayed its activity through its metabolites. In order to confirm this probability, however, atractylon, β-eudesmol, and hinesol were first treated with microsomes, and the reaction mixtures were assayed for antihepatotoxic activity. None of the metabolic mixtures inhibited CCl_4-induced injury in primary cultured rat hepatocytes, a fact which demonstrated that atractylon, β-eudesmol, and hinesol are effective *per se*.

It is now generally accepted that, *in vivo*, the hepatotoxicity of CCl_4 is dependent on its metabolism to CCl_3 radical by NADPH-cytochrome P-450 enzyme system of the liver cell endoplasmic reticulum and that the subsequent covalent binding of CCl_3 radical to cellular macromolecules and peroxidation of phospholipids of the endoplasmic reticulum membrane are the main sequence to liver injury (8). Because atractylon contains a furan ring, it was considered to exert its antihepatotoxic activity as a radical scavenger. However, because furfuryl alcohol, a furan analog, did not show activity comparable to that of atractylon (table 2), the above postulated mechanism was excluded.

With interest from the standpoint of structure-activity relationship, the antihepatotoxic actions of some available derivatives of β-eudesmol and hinesol were determined. As a result it was revealed that (a) hydrogenation of the sesquiterpenoids

TABLE 2. Effect of constituents of *Atractylodes* rhizomes and their analogs on carbon tetrachloride- and galactosamine-induced cytotoxicity in primary cultured rat hepatocytes (n=3 dishes).

Substance	Dose (mg/ml)	GPT (%)	
		CCl_4	GalN
Control	—	100±4	100±2
Atractylon	0.01	67±3[a]	99±1
	0.1	53±6[a]	92±2
	1.0	25±2[b]	84±1[a]
Atractylenolide I	1.0	68±7	107±2
Atractylenolide II	1.0	74±1[a]	94±5
Atractylenolide III	1.0	103±5	97±4
β-Eudesmol	0.01	83±6	104±2
	0.1	70±4[a]	94±1
	1.0	53±2[b]	79±1[b]
Hinesol	0.01	79±6	101±1
	0.1	62±3[a]	99±1
	1.0	31±1[b]	73±2[b]
Dihydroeudesmol	1.0	58±2[b]	83±1[a]
3-Hydroxydihydroeudesmol	1.0	44±2[b]	82±2[a]
3-Oxodihydroeudesmol	1.0	71±3[a]	81±4
Dihydrohinesol	1.0	35±0[b]	67±3[b]
Hinesotriol	1.0	74±1[a]	70±5[a]
Furfuryl alcohol	0.01	102±2	
	0.1	101±3	
	1.0	90±1	

[a]Significantly different from the control, $p < 0.01$.
[b]Significantly different from the control, $p < 0.001$.

caused no apparent changes in activity, (b) introduction of a hydroxyl and a carbonyl at C-3 of dihydroeudesmol produced an increase and a decrease in activity, respectively, in the CCl_4-induced liver lesion model, although essentially no alterations were observed in the GalN-produced liver injury model and (c) oxidation of hinesol appreciably diminished the activity.

It was recently reported that the hexane extract and the essential oil of the rhizomes of *A. japonica* and the 50% methanol extracts of *A. ovata* and *A. japonica* exerted antihepatotoxic actions in CCl_4-induced liver lesion in rats and mice (9, 10), although the former two samples were inactive in GalN-produced liver damage in rats (9). Further, the active principle of the latter two samples was revealed to be atractylon by the *in vivo* model system of liver injury in mice (10), supporting the presently obtained results on atractylon by the *in vitro* assay method. While the 50% methanol extracts of the rhizomes of *A. lancea* and *A. lancea* var. *chinensis* were reported to exert no antihepatotoxic effects in CCl_4-intoxicated mice (10), this may be explained by the fact that the liver-protective activity of extracts of *A. lancea* and its varieties exhibited great variation (table 1).

It may be worthy to note that *Atractylodes* rhizomes, the active sesquiterpenoids of which amount to 3-6% (11), still showed no significant antihepatotoxic activity (table 1). This may suggest the presence of some other constituents that may be hepatotoxic principles.

EXPERIMENTAL

MATERIALS.—The natural sesquiterpenoids were isolated from the crude drug, and their derivatives were prepared from them (5-7).

DETERMINATION OF ANTIHEPATOTOXIC ACTIVITY.—Assay was carried out using CCl_4- and

GalN-induced cytotoxicity in primary cultured rat hepatocytes as described previously (3,4).

DETERMINATION OF INFLUENCE ON TRANSAMINASE ACTIVITY.—Atractylon, β-eudesmol, or hinesol (0.2 mg) was dissolved in dimethylsulfoxide (2.0 μl) and diluted with the culture medium (200 μl) to which a standard serum (2440 IU/liter, 50 μl) was added. After standing at room temperature for 0.5 h, GPT activity was determined.

MICROSOME TREATMENT.—Atractylon, β-eudesmol, or hinesol (0.01 mg) was incubated at 37° for 30 min in the phosphate buffer (pH 7.4, 0.14 ml) containing microsomal protein (0.17 mg) and the NADPH generating system (5 mM glucose-6-phosphate, 0.5 U glucose-6-phosphate dehydrogenase, 33 mM KCl, 8 mM $MgCl_2$, and 4 mM NADPH). The reaction mixture was then heated at 56° for 30 min and subjected to bioassay against CCl_4 hepatotoxicity.

STATISTICAL ANALYSIS.—The data are expressed in mean ± S.E., and statistical significances were evaluated by one-way analysis of variance.

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