Araloside A, an Antiulcer Constituent from the Root Bark of *Aralia elata*

Eun Bang LEE,*[,a] Oon Ja KIM,[a] Sam Sik KANG,[a] and Choonsik JEONG[b]

[a] *Natural Products Research Institute and College of Pharmacy, Seoul National University; 28 Yeongun-dong, Chongno-ku, Seoul 110–460, Korea, and* [b] *College of Pharmacy, Duksung Women's University; Seoul 132–714, Korea.*
Received August 25, 2004; accepted November 8, 2004

Araloside A, a potent inhibitor of gastric lesion and ulcer formation in rats, was isolated from the root bark of *Aralia elata* through a bioassay-guided separation procedure. The compound exhibited significant reduction of HCl·ethanol-induced gastric lesions and aspirin-induced gastric ulcers at oral doses of 50 and 100 mg/kg, respectively. These activities are comparable to those of cimetidine.

Key words araloside A; antiulcer activity; *Aralia elata*; Araliaceae

The bark and root bark of *Aralia elata* SEEM (Araliaceae) have been used as folk medicine in Oriental countries for the treatment of diabetes,[1,2] gastric ulcer,[2,3] and hepatitis[2] and in inflammatory diseases including rheumatoid arthritis.[2] Several bioactive constituents were reported from the plant, *i.e.*, hypoglycemic constituents,[4—7] inhibitors of ethanol absorption,[8,9] and cytoprotective saponins in CCl_4-induced hepatic injury.[10] In terms of antiulcer activity, the root extract was found to have antisecretory action[11] and to exhibit potent inhibition of gastric lesion formation in rats,[12] but the active compounds were not clarified. Miscellaneous constituents have also been reported.[13—17] The present study deals with isolation of an antiulcer compound from the root bark of *A. elata* through bioassay-guided separation.

MATERIALS AND METHODS

Plant Material The roots of *A. elata* were collected in the plant garden cultivated at the Natural Products Research Institute, Seoul National University, in October 2000 and a voucher specimen (No. 001005) is maintained.

Extraction and Fractionation The dried root bark (1.32 kg) was sliced and refluxed with 70% methanol solution for 4 h two times in a water bath. The solution was filtered through gauze and evaporated to remove methanol and water under a vacuum and then freeze-dried to give a powdered extract (360 g). The extract was dissolved in 10% methanol solution and partitioned in succession with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol to give a hexane-soluble fraction (20.4 g, 1.55%), chloroform-soluble fraction (14.4 g, 1.09%), ethyl acetate-soluble fraction (18.0 g, 1.36%), and butanol-soluble fraction (213.6 g, 16.2%). The remaining layer was evaporated to give a water fraction (72 g, 5.46%).

Isolation of the Main Constituent of the Butanol Fraction Part (200 g) of the butanol fraction was passed through a silica gel column to obtain 18 fractions, using a gradient elution with chloroform–methanol–water (7 : 3 : 1) system. Among them, only fraction 12 exhibited potent inhibition of aspirin-induced gastric ulcer formation. Active fraction 12 was separated on a silica gel column using ethyl acetate saturated with water-methanol (gradient) to obtain the active constituent, which was identified as araloside A (chikusetsusaponin IV), mp 203—206 °C, using physicochemical and spectral methods[15] (Chart 1).

Animals Male Sprague-Dawley rats (180—210 g) and male ICR mice (23—30 g) were used in the experiments. All animals were housed in a temperature-controlled room with a 12-h light period. They were fed commercial solid food (Samyang Yuji Co. Ltd., Seoul) and tap water *ad libitum*. The test materials were suspended in 2% carboxymethylcellulose solution and given in a volume of 0.2 ml/100 g body weight. The doses of the test materials were chosen based on the yields obtained from the original extract or fractions.

HCl·Ethanol-Induced Gastric Lesion in Rats As previously described by Mizui and Dodeuchi,[18] the rats were deprived of food but allowed free access to drinking water for 24 h and 1.5 ml of 60% ethanol in HCl 150 mM was given orally to each rat. One hour later, the animals were killed under ether anesthesia and the stomachs were removed and examined for lesions in the glandular portion. The lesion index was expressed as the length (mm) of lesions. The test sample and reference drug were given orally 30 min before giving HCl–ethanol solution.

Aspirin-Induced Gastric Ulcer in Pylorus-Ligated Rats According to the method of Okabe *et al.*,[19] the rats were fasted for 24 h, the pylorus was ligated under ether anesthesia, and the test sample was given intraduodenally (i.d.). Ten minutes later, 100 mg/kg of aspirin suspended in 5% gum acacia solution was given. Seven hours after aspirin administration, the animals were killed under ether anesthesia and the stomachs were removed and examined for the ulcers. The ulcer index was expressed as length (mm) of ulcers.

Water Immersion Stress-Induced Ulcer Induction in Rats According to the method of Takagi and Okabe,[20] the rats were immobilized in stress cages that were immersed in a water bath at 22±2 °C to the level of the rat xiphoid for 10 h. The animals were killed 7 h after being subjected to the

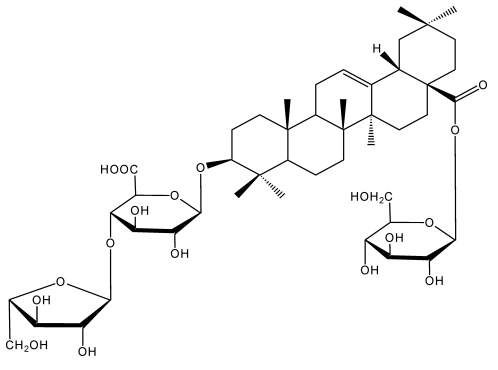

Chart 1. Chemical Structure of Araloside A

* To whom correspondence should be addressed. e-mail: eblee@snu.ac.kr

stress and their ulcer indices were expressed as the area (mm^2) of the ulcers. The test sample was given orally 30 min before immersing the animals.

Shay Ulcers in Rats The rats were deprived of food for 24 h, the stomach was incised, and the pylorus was ligated under ether anesthesia as described by Shay *et al.*[21] Fourteen hours later, the animals were killed and the stomach was removed, excised, and treated with formalin solution. The ulcer index was determined based on the severity of lesions found in the glandular portion: 0, no lesion; 1, bleeding and slight lesions; 2, moderate lesions; 3, severe lesions; and 4, perforated ulcers. The test material was given intraduodenally immediately after pylorus ligation.

Gastric Secretion in Rats According to the method of Shay *et al.*,[21] the abdomen was incised and the pylorus was ligated under ether anesthesia. Four hours after intraduodenal administration of the sample, the animals were killed, the stomach was removed, and the gastric fluid collected. Following centrifugation of the fluid at 3000 rpm for 10 min, the volume of gastric juice and pH were measured. The acidity was determined by titrating with 0.05 N NaOH, and acid output (μEq/h) was calculated.

Acute Toxicity The acute toxicity of araloside A was evaluated after 1-week oral administration to male mice, and the LD_{50} value was calculated according to the method of Litchfield and Wilcoxon[22] with a 95% confidence limit.

Statistical Analyses All data are expressed as means±S.E.M. Statistical analysis was performed using analysis of variance followed by Dunnett's *t*-test. Values of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The hexane, chloroform, ethyl acetate, butanol, and water fractions were tested in HCl·ethanol-induced gastric lesions. As shown in Fig. 1, the butanol-soluble portion showed the most potent activity at 200 mg/kg *p.o.* and it is assumed that its action is nearly equipotent to that of the original methanol extract at 700 mg/kg *p.o.* This indicates that most of the active constituent(s) might be contained in the butanol fraction, even if the chloroform and water fractions have weak activity. It is interesting that the activity of the butanol-soluble fraction at the dose of 200 mg/kg *p.o.* is much more potent than that of cimetidine 100 mg/kg.

In a further bioassay at two dose levels, the butanol fraction showed potent dose-dependent inhibition of HCl·ethanol-induced gastric lesion formation, and the activity was more potent than that of cimetidine (Fig. 2A). The butanol-soluble fraction also significantly reduced aspirin-induced gastric ulcer formation in pylorus-ligated rats at doses of 200 and 500 mg/kg i.d. (Fig. 2B).

The butanol fraction was subjected to silica gel column chromatography to give 18 subfractions in the yields shown in Table 1. The activity of these subfractions were tested against aspirin-induced ulcer formation. Each dose of the subfractions was chosen proportionally to the yield of each subfraction obtained in the chromatographic isolation procedure. The results are shown in Table 2. Only one subfraction of fraction 12 showed significant inhibitory activity at a dose of 83.9 mg/kg i.d. (72% inhibition), which is nearly equipotent to that of cimetidine. However, another eight fractions tested were found to lack the activity. Five subfractions (nos. 6, 10, 11, 14, 15) did not need to be tested, because each was a mixture of the adjacent subfractions on silica gel TLC plates, and four fractions (nos. 1, 2, 9, 18) could not be tested, because the quantities obtained from the butanol fraction were too small. Therefore only the active subfraction of fraction 12 was further fractionated by silica gel column chromatography, yielding araloside A (chikusetsusaponin IV, 0.9% from the dried plant).[15] The activity of araloside A

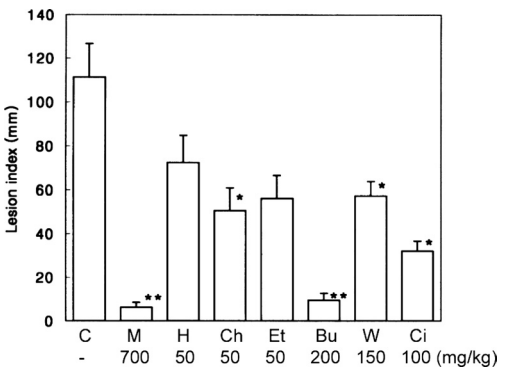

Fig. 1. Effects of the Methanol Extract and Its Fractions of *Aralia elata* on HCl·Ethanol-Induced Gastric Lesion in Rats

$*p<0.05$, $**p<0.01$, significantly different from the control group. C, control; M, methanol extract; H, hexane fraction; Ch, chloroform fraction; Et, ethyl acetate fraction; Bu, butanol fraction; W, water fraction; Ci, cimetidine.

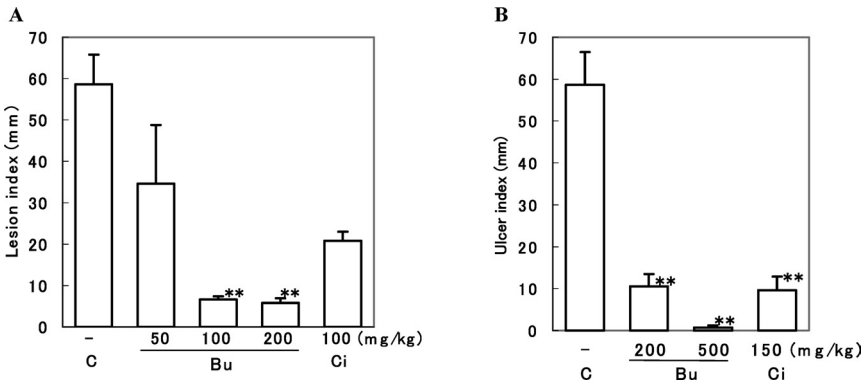

Fig. 2. Effects of the Butanol Fraction of *Aralia elata* on HCl·Ethanol-Induced Gastric Lesion (A) and Aspirin-Induced Gastric Ulcer (B) Formation in Rats

$**p<0.01$, significantly different from the control group. C, control; Bu, butanol fraction. Ci, cimetidine.

Table 1. Fractionation of Butanol Portion on Column Chromatography

Subfraction No.	Yield (g)	Yield (%)
1	0.86	0.5
2	1.14	0.6
3	2.64	1.4
4	5.77	3.1
5	5.20	2.8
6	4.67	2.6
7	8.21	4.4
8	10.29	5.6
9	0.82	0.4
10	2.93	1.6
11	6.36	3.4
12	29.28	15.8
13	39.26	21.2
14	4.11	2.2
15	6.77	3.7
16	18.52	10.1
17	36.46	19.7
18	1.63	0.9
Total	184.92	100

Table 2. Effect of Butanol Portion of *Aralia elata* on Aspirin-Induced Gastric Ulcer in Rats

Fraction	Dose (mg/kg, *i.d.*)	No. of animals	Ulcer index (Mean±S.E.)	Inhibition (%)
Control	—	7	38.3±6.8	—
No. 3	8.0	7	44.0±7.9	−14.9
No. 4	16.6	7	41.0±11.2	−7.1
No. 5	14.9	7	39.0±7.5	−1.8
Cimetidine	100.0	7	15.4±5.2*	59.8
Control	—	7	34.4±7.1	—
No. 7	23.5	7	37.0±7.6	−7.6
No. 8	29.5	7	41.1±7.4	−19.5
No.12	83.9	7	9.7±4.8*	71.8
Cimetidine	100.0	7	6.0±3.3**	82.6
Control	—	7	47.3±9.6	—
No.13	118.0	7	47.1±5.5	0.4
No.16	53.1	7	48.3±6.7	−2.1
No.17	104.5	7	45.9±8.2	3.0
Cimetidine	100.0	7	3.3±1.1**	93.0

Significantly different from the control group (∗$p<0.05$, ∗∗$p<0.01$).

against HCl·ethanol-induced gastric lesion and aspirin-induced gastric ulcer formation at the two dose levels of 50 and 100 mg/kg *p.o.* was determined in rats. At the dose of 100 mg/kg *p.o.* the compound exhibited significant inhibitory activity in the gastric lesion model as compared with the control group (Table 3). The effects of araloside A were similar to those of cimetidine. At the lower dose of 50 mg/kg *p.o.* it did not show any significant inhibition, as previously reported[23] at the same dose in a model of absolute ethanol-induced gastric lesion formation. Araloside A at a dose of 100 mg/kg i.d. showed significant inhibition of aspirin-induced ulcer in pylorus-ligated rats, which had nearly the same potency as cimetidine 100 mg/kg (Table 4).

Araloside A inhibited stress-induced ulceration in a dose-dependent manner. At the doses of 50 and 100 mg/kg *p.o.*, it showed significant inhibition, as shown in Table 5. The compound has more potent action than that of cimetidine.

Araloside A 50 and 100 mg/kg i.d. inhibited Shay ulceration in a dose-dependent manner and the difference compared with the control group was significant (Table 6).

Table 3. Effect of Araloside A on HCl·Ethanol-Induced Gastric Lesion in Rats

Treatment	Dose (mg/kg, *p.o.*)	No. of animals	Lesion index (Mean±S.E.)	Inhibition (%)
Control	—	7	61.9±10.5	—
Araloside A	50	7	45.0±8.3	27.3
	100	7	30.1±5.5*	51.4
Cimetidine	100	7	28.5±3.3*	54.0

Significantly different from the control group (∗$p<0.05$).

Table 4. Effect of Araloside A on Aspirin-Induced Gastric Ulcer in Rats

Treatment	Dose (mg/kg, *i.d.*)	No. of animals	Ulcer index (Mean±S.E.)	Inhibition (%)
Control	—	7	41.9±10.0	—
Araloside A	50	7	21.0±4.3	49.9
	100	7	8.1±8.0*	80.7
Cimetidine	100	7	6.5±2.3*	84.5

Significantly different from the control group (∗$p<0.05$).

Table 5. Effect of Araloside A on Water-Immersion Stress Ulcer in Rats

Treatment	Dose (mg/kg, *p.o.*)	No. of animals	Ulcer index (Mean±S.E.)	Inhibition (%)
Saline	—	8	71.5±5.8	—
Araloside A	50	7	30.5±8.6*	57.8
	100	8	11.2±6.3**	84.3
Cimetidine	100	8	18.3±5.8**	74.4

Significantly different from the saline group (∗$p<0.05$, ∗∗$p<0.01$).

Table 6. Effect of Araloside A on Shay Ulcer in Rats

Treatment	Dose (mg/kg, *i.d.*)	No. of animals	Ulcer index (Mean±S.E.)	Inhibition (%)
Saline	—	8	2.3±0.6	—
Araloside A	50	8	0.8±0.2*	65.2
	100	8	0.6±0.4*	73.9
Cimetidine	100	8	0.0±0.0**	100.0

The animals were sacrificed 10 h after pylorus-ligation. Significantly different from the saline group (∗$p<0.05$, ∗∗$p<0.01$).

Araloside A 50 and 100 mg/kg i.d. inhibited the secretion of gastric juice, acid secretion, and total acid output in a dose-dependent manner. It also lowered the pH value in a similar pattern (Table 7). Thus araloside A may exert antiulcer activity *via* inhibition of gastric acid output.

The acute toxicity of araloside A expressed as the LD_{50} value in male mice was 3.22 (2.09—4.96) g/kg *p.o.*, indicating that the toxicity is very weak.

In conclusion, our results indicate that araloside A is the main constituent with antiulcer action of the root bark of *A. elata*, the relative potency of araloside A and cimetidine do not differ greatly, and its antiulcer action may be due to the inhibition of gastric acid secretion. The ethnomedical uses of *A. elata* in treating gastric ulcerative symptoms may be attributed to the effects of this constituent.

Table 7. Effect of Araloside A on Gastric Secretion in Rats

Treatment	Dose (mg/kg, *i.d.*)	No. of animals	Gastric juice volume (ml)	pH	Acidity (μEq/ml)	Total acid output (μEq/4 h)
Saline	—	7	7.1±0.9	1.50±0.05	116.8±5.0	822.9±93.4
Araloside A	25	7	4.7±0.7	1.57±0.08	112.3±4.8	536.9±94.9
	50	7	3.2±0.2*	2.21±0.38	90.2±6.5*	293.6±33.8*
	100	7	1.4±0.2*	3.89±0.72*	76.8±9.0*	112.7±24.5*
Cimetidine	100	7	2.6±0.6*	5.21±0.93*	62.2±8.6*	194.3±70.0*

The animals were sacrificed 4 h after pylorus-ligation. Significantly different from the saline group ($*\ p<0.05$).

REFERENCES

1) Yook C. S., "Medicinal Plants of Korea," Jinmyung Publishing Co., Ltd., Seoul, 1981, p. 272.
2) Namba T., "The Encyclopedia of Wakan-Yaku with Color Pictures," Vol. II, Hoikusha Publishing Co., Ltd., Osaka, 1980, p. 180.
3) Munwha Broadcast Corporation (ed.), "Korean Folk Medicinal Therapeutics," Keumbak Publishing. Co., Ltd., Seoul, 1987, p. 57.
4) Kim O. K., Lee E. B., Kang S. S., *Kor. J. Pharmacogn.*, **24**, 219—222 (1993).
5) Yoshikawa M., Matsuda H., Harada E., Murakami T., Wariishi N., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **42**, 1354—1356 (1994).
6) Yoshikawa M., Yosizimi S., Ueno T., Matsuda H., Murakami T., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **43**, 1878—1882 (1995).
7) Yoshikawa M., Murakami T., Harada E., Murakami N., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **44**, 1923—1927 (1996).
8) Yoshikawa M., Harada E., Matsuda H., Murakami T., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **41**, 2069—2071 (1993).
9) Yoshikawa M., Murakami T., Harada E., Murakami N., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **44**, 1915—1922 (1996).
10) Saito S., Ebashi J., Sumito S., Fukumoto T., Nagamura Y., Nishida K., Ishiguro I., *Chem. Pharm. Bull.*, **41**, 1395—1401 (1993).
11) Hernandez D. E., Haneke J. L., Wikman G. J., *J. Ethnopharmacol.*, **23**, 109—114 (1988).
12) Lee E. B., Jung C. S., *Yakhak Hoeji*, **37**, 581—590 (1993).
13) Satoh Y., Sakai S., Katsumata M., Nagasao M., Miyakoshi M., Ida Y., Shoji J., *Phytochemistry*, **36**, 147—152 (1994).
14) Kang S. S., Kim J. S., Kim O. K., Lee E. B., *Arch. Pharm. Res.* (Seoul), **16**, 104—108 (1993).
15) Kang S. S., Kim J. S., Lee E. B., *Int. J. Pharmacogn.*, **34**, 119—123 (1996).
16) Kuang H., Sun H., Zhang N., Okada Y., Okuyama T., *Chem. Pharm. Bull.*, **44**, 2183—2185 (1996).
17) Kang S. S., Kim J. S., Xu Y. N., Kim Y. H., *J. Nat. Prod.*, **62**, 1059—1060 (1999).
18) Mizui T., Dodeuchi K., *Jpn. J. Pharmacol.*, **33**, 939—945 (1983).
19) Okabe S., Takeuchi K., Nakamura K., Takagi K., *Jpn. J. Pharmacol.*, **34**, 363—371 (1984).
20) Takagi K., Okabe S., *Jpn. J. Pharmacol.*, **18**, 9—18 (1968).
21) Shay H., Komarov S. A., Fels S. S., Meranze D., Guenstein M., Siplet H., *Gastroenterology*, **5**, 43—51 (1945).
22) Litchfield T., Wilcoxon A., *J. Pharmacol. Exp. Ther.*, **96**, 99—111 (1949).
23) Matsuda H., Li Y., Murakami T., Yamahara J., Yoshikawa M., *Life Sci.*, **63**, 245—250 (l998).