Comparison of Spasmolytic Action Between *Ligusticum wallichi* Franch and *Cnidium officinale* Makino

By

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I). INTRODUCTION:

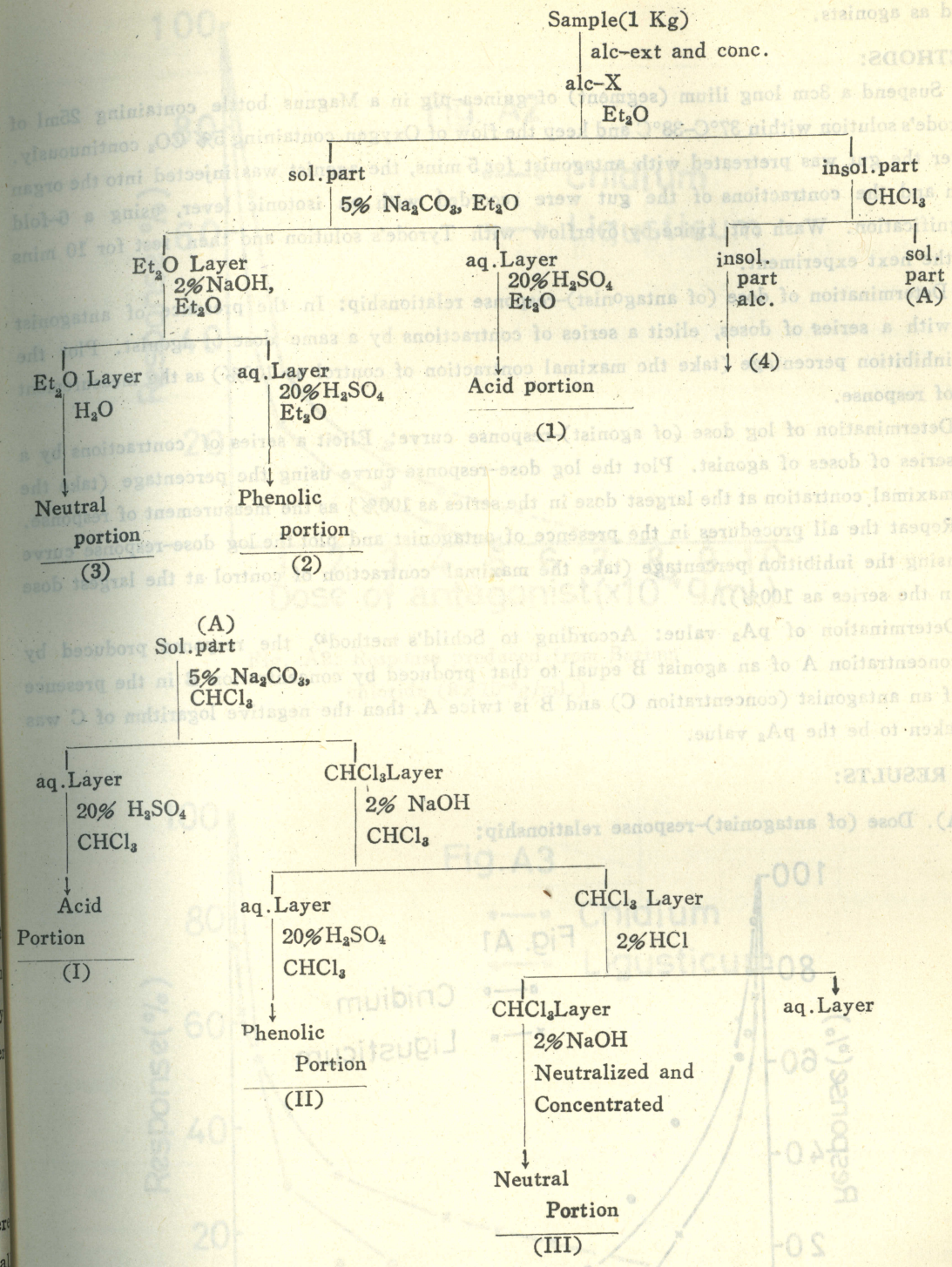
Both drugs of the Ligusticum and the Cnidium have been used as sedatives and antispasmodics in China for a long time. In 1935[1], the Ligusticum was confirmed that would be able to contract the pregnant uterus in a small dose, and paralyze the normal uterus and intestine in a large dose. It may prevent abortion in the continuous injection of its crude extract. It may paralyze sympathetic nerve, dilatate blood vessel and cause hypotension. It may potentiate the hypotensive action of reserpine. It may inhibit cerebral activity and cause sedation of animal. In 1957[1], it was obtained several constituents. One of these components, ferulic acid was confirmed to be not a primary principle on spasmolytic action in our laboratory. However, the neutral portion of ether or chloroform extract was confirmed to be the primary portion in this paper. In 1963[2a] and 1967[2a], Dr. H. Mitsuhashi obtained several principles, including butylidenphthalide, ligustilide, cnidilide, and neo-cnidilide from the neutral portion of Cnidium officinale Makino. These principles own some spasmolytic action. In our data, there is a very important fact that the papaverine-like action of the Ligusticum is very stronger than that of the Cnidium but the atropine-like action and antihistamine-like action of the Ligusticum are not significantly different from those of the Cnidium. It is very interesting whether the Ligusticum has other principles which are different from the Cnidium.

II). EXPERIMENTAL METHODS AND MATERIALS:

MATERIALS:

The dry rhizomes of Ligusticum wallichi Franch and Cnidium officinale Makino were purchased from Taipei market. Ten grams of each sample were weighed and cut into small pieces and then extracted with 100 ml. of 95% ethyl alcohol for 48 hrs in water bath. Finally made up the ethyl alcohol to the concentration of 10g/ml. This concentration was used for the determination of dose (of antagonist)-response relationship and log dose (of agonist)-response curve.

Other purchased rhizomes were divided into several portions, according to the following method:



Each portion was evaporated and weighed for making up the concentration of 5mg/ml with 95% ethyl alcohol. In addition to each portion, the alcoholic crude extracts of the Ligusticum and the Cnidium were also evaporated and weighed for making up the same concentration with 95% ethyl alcohol. This concentration was used for the determination of pA_2.

Ferulic acid was purchased from Sigma Chemical Company, U.S.A. Acetylcholine (OVISOT, Tokyo Daiichi Seiyaku Co., LTD, Japan), Barium chloride (Wako Pure Chemical Industries, LTD, Osaka, Japan), and Histamine diphosphate (Mann Research Laboratories Inc., N.Y.) were

used as agonists.

METHODS:

Suspend a 3cm long ilium (segment) of guinea-pig in a Magnus bottle containing 25ml of Tyrode's solution within 37°C–38°C and keep the flow of Oxygen containing 5% CO_2 continuously. After the gut was pretreated with antagonist for 5 mins, the agonist was injected into the organ bath and the contractions of the gut were recorded with an isotonic lever, using a 6-fold magnification. Wash out twice by overflow with Tyrode's solution and then rest for 10 mins for the next experiment.

A) Determination of dose (of antagonist)–response relationship: In the presence of antagonist with a series of doses, elicit a series of contractions by a same dose of agonist. Plot the inhibition percentage (take the maximal contraction of control as 100%) as the measurement of response.

B) Determination of log dose (of agonist)–response curve: Elicit a series of contractions by a series of doses of agonist. Plot the log dose–response curve using the percentage (take the maximal contration at the largest dose in the series as 100%) as the measurement of response. Repeat the all procedures in the presence of antagonist and plot the log dose–response curve using the inhibition percentage (take the maximal contraction of control at the largest dose in the series as 100%).

C) Determination of pA_2 value: According to Schild's method[8], the response produced by concentration A of an agonist B equal to that produced by concentration B in the presence of an antagonist (concentration C) and B is twice A, then the negative logarithm of C was taken to be the pA_2 value.

III). RESULTS:

A). Dose (of antagonist)–response relationship:

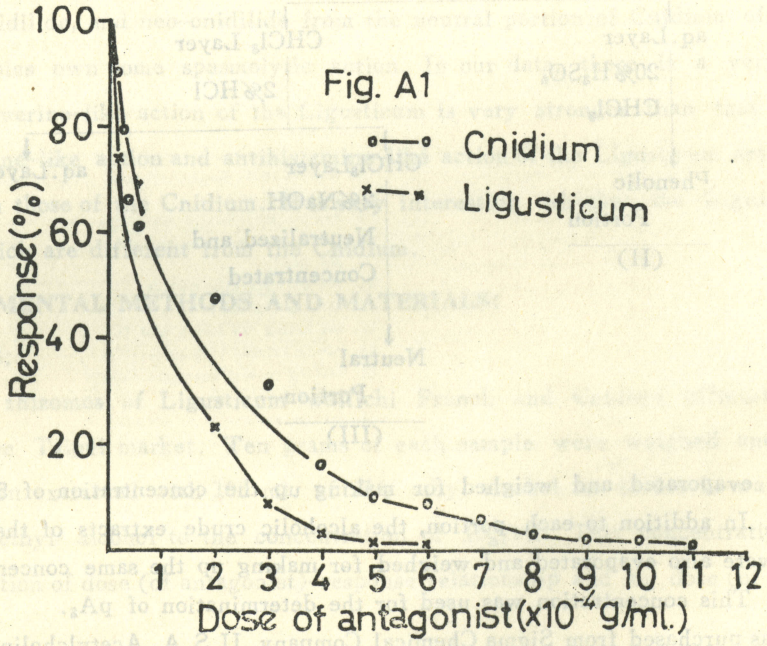


Fig. A1: Response produced from Acetylcholine (16×10^{-8}g/ml.)

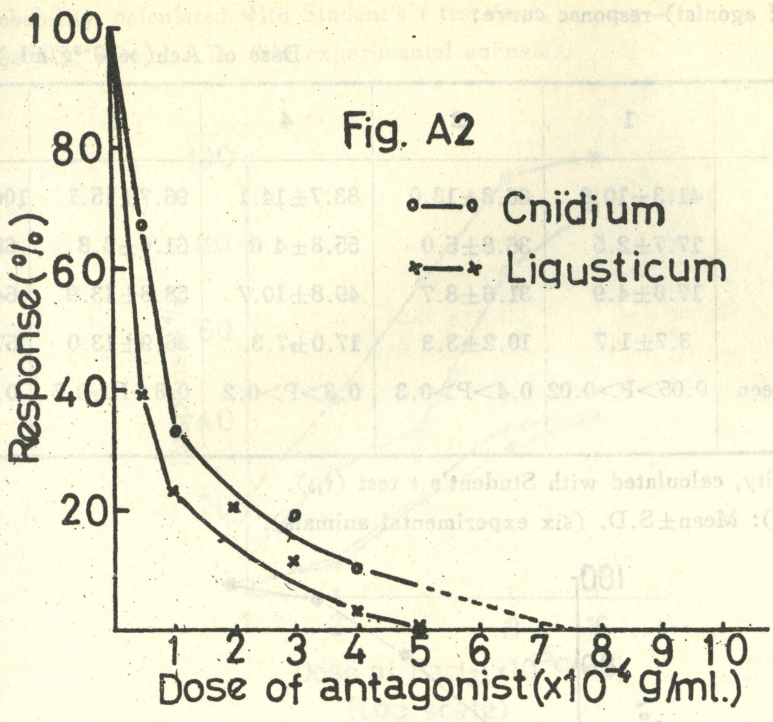


Fig. A2: Response produced from Barium chloride (8×10^{-5}g/ml.)

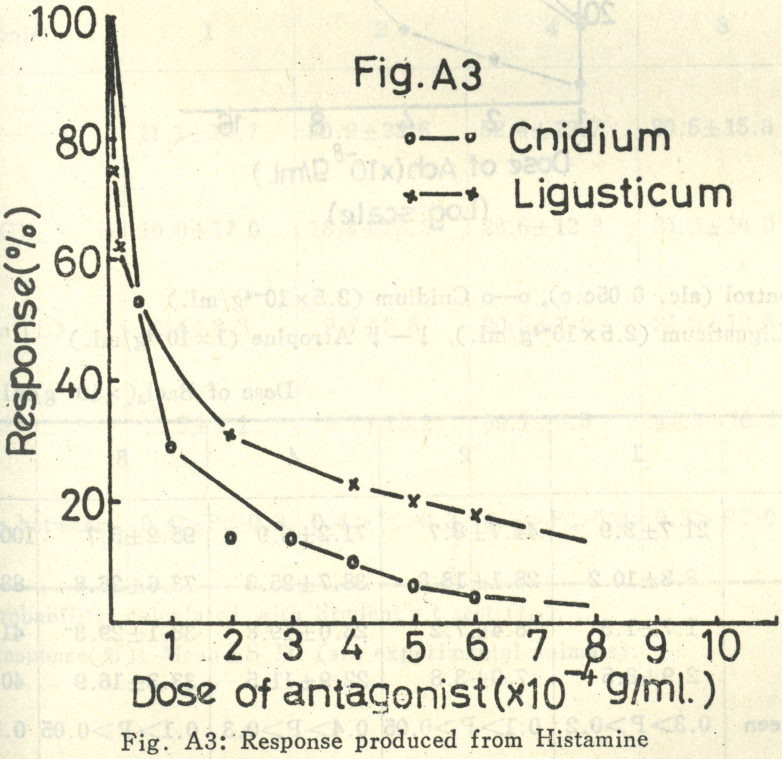


Fig. A3: Response produced from Histamine diphosphate (8×10^{-8}g/ml.)

B). Log dose (of agonist)–response curve:

Response(%) Dose of Ach($\times 10^{-8}$g/ml.)

Antagonist	1	2	4	8	16
Control	41.3±10.8	66.3±13.0	83.7±14.1	96.7±15.1	100.0±0.0
Cnidium (C)	17.7±2.5	36.8±5.0	55.8±4.6	61.0±8.8	65.8±9.8
Ligusticum (L)	17.0±4.9	31.6±8.7	49.8±10.7	58.8±13.9	64.0±5.0
Atropine	3.7±1.7	10.2±3.3	17.0±7.3	36.9±13.0	57.6±11.3
Difference between C&L	0.05>P>0.02	0.4>P>0.3	0.3>P>0.2	0.8>P>0.7	0.7>P>0.6

Note: P: probability, calculated with Student's t test (t_{10}).

Response(%): Mean±S.D. (six experimental animals).

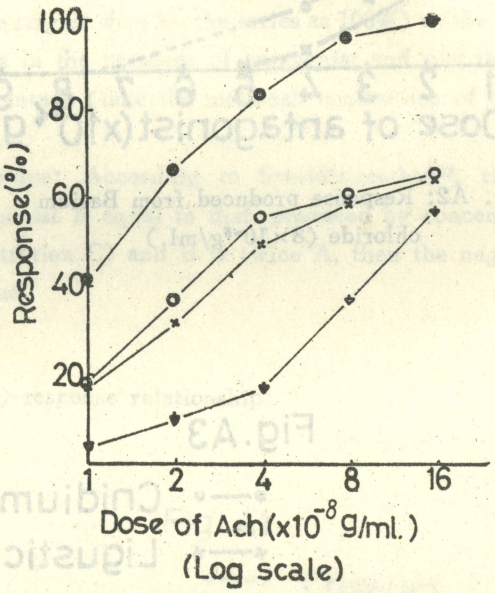


Fig. B1: ·—· Control (alc. 0.05c.c), o—o Cnidium (2.5×10^{-4}g/ml.)
×—× Ligusticum (2.5×10^{-4}g/ml.), ↓—↓ Atropine (1×10^{-4}g/ml.)

Response(%) Dose of $Bacl_2$($\times 10^{-5}$g/ml.)

Antagonist	1	2	4	8	16
Control	21.7±3.9	44.7±8.7	71.2±3.9	95.2±5.7	100.0±0.0
Cnidium (C)	8.8±10.2	28.1±18.8	38.7±25.3	73.6±26.8	88.5±33.7
Ligusticum (L)	1.7±1.3	8.4±7.2	23.0±19.8	38.1±29.3	41.8±31.6
Papaverine	2.9±3.5	7.9±3.8	22.9±11.5	33.3±16.9	40.9±20.2
Difference between C&L.	0.3>P>0.2	0.1>P>0.05	0.4>P>0.3	0.1>P>0.05	0.1>P>0.05

Note: P: probability, calculated with Student's t test (t_{10}).
Reponse(%): Mean±S.D. (six experimental animals).

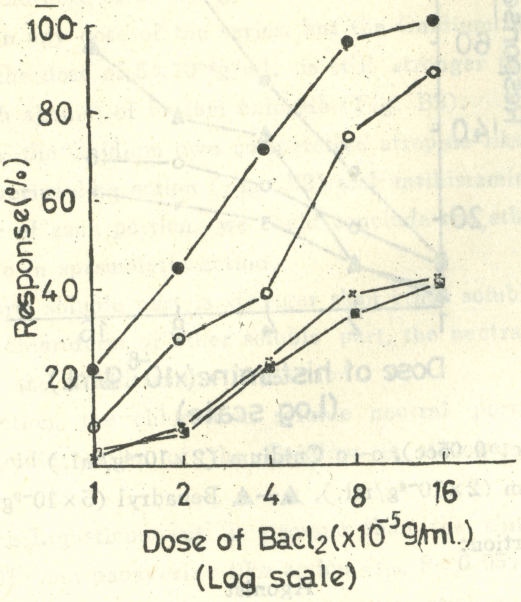


Fig. B2: ·—· Control (alc. 0.05), o—o Cnidium (1×10^{-3}g/ml.)
×—× Ligusticum (5×10^{-4}g/ml.) ■—■ Papaverine (5×10^{-6}g/ml.)

Response(%) Dose of Histamine ($\times10^{-8}$g/ml.)

Antagonist	1	2	4	8	16
Control	11.1±12.7	30.9±23.6	52.4±19.7	80.5±15.3	100.0±0.0
Cnidium (C)	10.0±17.0	18.4±23.3	28.6±12.3	34.3±16.0	35.6±13.0
Ligusticum (L)	3.4±8.3	9.7±2.8	20.6±1.2	24.5±12.5	27.8±14.6
Benadryl	2.5±5.1	9.7±11.2	39.7±9.5	44.3±26.4	61.4±27.1
Difference between C&L	0.4>P>0.3	0.4>P>0.3	0.3>P>0.2	0.3>P>0.2	0.4>P>0.3

Note: P: probability, calculated with Student's t test (t_{10}).
Response(%): Mean±S.D. (six experimental animals).

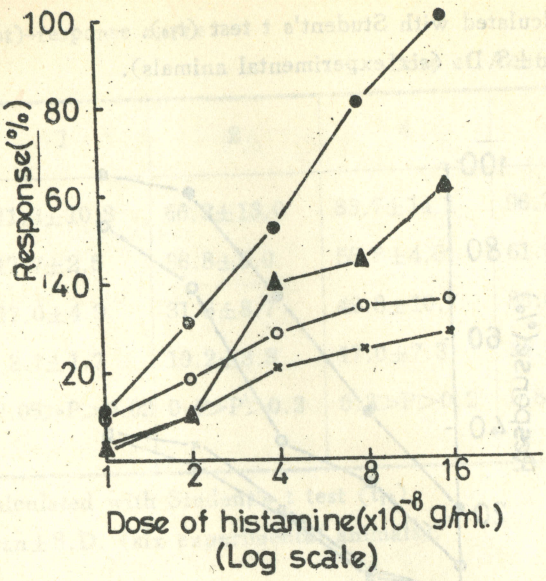


Fig. B3: •—• Control (alc. 0.05cc), o—o Cnidium (2×10^{-4}g/ml.)
×—× Ligusticum (2×10^{-4}g/ml.), ▲—▲ Benadryl (5×10^{-9}g/ml.)

C). pA_2 value of each portion:

Antagonist	Agonist		
	Ach	$BaCl_2$	Histamine
Cnidium(1)	4.5±0.32	4.5±0.10	4.6±0.86
(2)	5.3±0.55	4.8±0.10	5.1±0.23
(3)	4.8±0.75	4.6±0.23	4.5±0.26
(4)	4.3±0.46	4.6±0.28	4.1±0.61
(I)	6.1±0.90	4.9±0.29	4.8±0.29
(II)	5.1±0.57	4.9±0.67	4.8±0.31
(III)	5.1±0.53	5.0±0.46	4.9±0.16
Ligusticum (1)	4.6±0.29	4.5±0.17	4.5±0.38
(2)	4.7±0.12	4.7±0.26	4.8±0.31
(3)	4.9±0.45	4.8±0.17	5.2±0.56
(4)	4.1±0.26	4.4±0.00	3.7±0.16
(I)	5.4±0.38	4.8±0.94	4.5±0.36
(II)	5.8±1.36	4.6±0.21	5.3±1.04
(III)	6.1±0.90	5.5±0.47	6.0±0.00
Cnidium ext.	4.7±0.06	4.6±0.45	4.5±0.63
Ligusticum ext.	5.1±0.08	5.4±0.30	4.6±0.58
Ferulic acid	6.5±0.89	4.2±0.32	3.7±0.42
Atropine	8.7±0.27		
Papaverine		5.6±0.4	
Benadryl			7.9±0.40

Note: 1). Six experimental animals were used.
2). Values are expressed by mean±S.D.

IV). DISCUSSION:

1. The Ligusticum is not significantly stronger than the Cnidium in the atropine-like and antihistamine-like action. (Fig. B1 and Fig. B3).
2. The Ligusticum in the dose of 1×10^{-3}g/ml. Paralyzes the ileum of guinea-pig with agonist of barium chloride in any dose of the series, but the Cnidium does not.
3. The Ligusticum in the dose of 5×10^{-4}g/ml. is still stronger than the Cnidium in the dose of 1×10^{-3}g/ml. with agonist of barium chloride (Fig. B2).
4. The Ligusticum and the Cnidium own competetive atropine-like action (Fig. B1), but own oncompetetive papaverine-like action (Fig. B2) and antihistamine-like action (Fig. B3).
5. From the pA_2 value of each portion, we could conclude the ether and chloroform insoluble part is less effective in spasmolytic action.
6. In general, chloroform soluble part is stronger than ether soluble part.
7. In each portion of chloroform or ether soluble part, the neutral portion is the strongest in the papaverine-like and antihistamine-like action.
8. In atropine-like action, the chloroform soluble neutral portion of the Ligusticum is the strongest, but the acidic portion of the Cnidium is stronger than other portion of the Cnidium itself.
9. From pA_2 value, the Ligusticum ext. is stronger than the Cnidium ext. in atropine-like action (t_{10}, $P<0.001$) and papaverine-like action (t_{10}, $P<0.05$).
10. Ferulic acid owns less effects in the papaverine-like action and antihistamine-like action, but owns some atropine-like action.
11. Taking antilogarithm of the difference between pA_2 of the Ligusticum extract and the Cnidium extract, we found the Ligusticum would be at least six-folds of the Cnidium-action, but the Ligusticum was only twice of the Cnidium in atropine-like action, and the Ligusticum was not significantly different from the Cnidium. in antihistamine-like action.

V). REFERENCES:

1). Kun-Ying Yen, Pharmacology of Common Chinese Medicine, National Research Institute of Chinese Medicine, p. 17-18, 1970.

2). H. Mitsuhashi, T. Muramatsu, U. Nagai, T. Nakano, K. Ueno: Chem. Pharma. Bull. (Tokyo) **11**, 1317, 1963; H. Bohrmann, E. Stahl, H. Mitsuhashi, Ibid., **15**, 1606, 1967.

3). H. O. Schild, Pharmacological reviews, **9**, p. 242, 1957.

SUMMARY

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Both drugs of the Ligusticum and the Cnidium have been used as sedatives and antispasmodics in China for a long time. In 1957, from the Ligusticum, it was obtained several constituents. One of these components, ferulic acid was confirmed to be not the primary principle on spasmolytic action in our laboratory. From the pA_2 value, it showed that the primary priciple should

be in the neutral portion of ether and chloroform extract. In our data, there was a very important fact that the papaverine-like action of the Ligusticum was very stronger than that of the Cnidium, but the atropine-like and antihistamine-like action of the Ligusticum were not significantly different from those of the Cnidium. From pA_2 value, we found the Ligusticum would be at least six-folds of the Cnidium in papaverine-like action. In atropine-like action, the Ligusticum was only twice of the Cnidium and in antihistamine-like action, the Ligusticum was not significantly the Cnidium. It is very interesting whether the Ligusticum has other principles which are different from the Cnidium. In addition, the Ligusticum and the Cnidium own competetive atropine-like action, but own non-competetive papaverine-like and antihistamine-like action will be noted.

ACKNOWLEDGEMENTS

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中 文 摘 要

川芎與日芎抗痙作用之比較

柯文昌，王耀東

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川芎 (Ligusticum wallichi Franch) 及日芎 (Cnidium officinale Makino) 自古以來皆用做抗痙、鎮靜劑。1957年川芎被證明內含ferulic acid有抗痙作用，然按本文實驗結果證明 ferulic acid 僅略具 Atropine-like action，至於 Papaverine-like 及 antihistamine-like action 無任何作用可言。由本實驗結果顯示其主要抗痙成分應在乙醚、氯仿可溶之中性部。

由 PA_2 值取其抗對數 (antilogarithms) 即可算出川芎之 Papaverine-like action 有日芎之六倍，而 Atropine-like action 却只有兩倍，至於 Antihistamine-like acton兩者並無區別。

川芎與日芎同具競爭性的 atropine-like action，但二者却具非競爭性的 Papaverine-like 及 antihistamine-like action。

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