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Spasmolytic Substances from *Cimicifuga dahurica* Maxim.[1]

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The search for spasmolytic principals of *Cimicifuga dahurica* Maxim. (Ranunculaceae) with the guidance of the intestinal action resulted in the isolation of two active substances, visamminol and visnagin, in addition to isoferulic acid and norvisnagin. Norvisnagin was first isolated from natural source. Pharmacological estimations using guinea pig jejunum revealed that the spasmolytic actions of visamminol and visnagin were one-third and one-tenth that of papaverine hydrochloride, respectively. Norvisnagin showed inactivity.

The rhizoma of the genus *Cimicifuga* (Ranunculaceae) have been used as an antipyretic, an analgesic and a spasmolytic in Japanese and Chinese folk medicines.[3,4] As another application, the rhizoma have been used as a folkloric gynecological remedy for the metroptoses.[4]

The first pharmacological study on extracts of *Cimicifuga dahurica* Maxim. has been shown that its extracts have had sedative and hypotensive effects as well as an anticonvulsant effect against camphor or strychnine in mice.[5] So far, no information is available about the active constituents, though several phenolic acids, triterpenes and saponin were isolated from this plant.[6,7]

The present paper deals with the isolation of spasmolytic substances from the rhizoma of *C. dahurica* with the guidance of the intestinal action. All spasmolytic effects were evaluated on isolated jejunum of guinea pig using acetylcholine chloride, histamine hydrochloride and barium chloride as spasmogens.

The 50% methanol-extract of the rhizoma prevented contractions caused by above three spasmogens. The extract was repeatedly macerated with chloroform and chloroform-soluble active substances were collected. The chloroform fraction responsible for the spasmolytic effect at 10^{-4}—10^{-5} g/ml concentrations relieved spasma caused by 10^{-7} g/ml of acetylcholine chloride to one-half (Fig. 1). The chloroform fraction after chromatography upon a silica gel column gave four crystalline substances, I (mp 228°), II (mp 154.5—156.5°), III (mp 154—156.6°) and IV (mp 141—143°). Substance I, $C_{10}H_{10}O_4$, was identical with isoferulic acid. II had the composition $C_{12}H_8O_4$ by elemental and mass spectral analyses. The ultraviolet (UV) spectrum of II showed maxima at 214 nm (log ε 4.66), 246 nm (sh., 4.86), 253 nm (4.92), 261 nm (sh., 4.76), 282 nm (4.16) and 337 nm (3.96) which was very similar to that of visnagin.[8] II had a broad hydroxyl band in its infrared (IR) spectrum at 2750 cm^{-1} indicative of strong intramolecular hydrogen bonding, in addition, II exhibited the positive Gibbs color test which indicated the presence of a phenolic group. The nuclear magnetic resonance (NMR)

1) This paper is Part X of "Studies on the Constituents of *Cimicifuga* spp." Part IX: G. Kusano and T. Takemoto, *Yakugaku Zasshi*, **95**, 1133 (1975).
2) Location: *Aobayama, Sendai.*
3) "Iconographic Cormophytorum Sinicorum", Tomus I, ed. by Chinese Academy of Sciences, Scientific Publisher, Peking, 1972, p. 664.
4) "Chuyaku Shi, Vol. I", ed. by Chinese Academy of Medical Scieuces, *et el.*, People's Hygiene Publisher, Peking, 1961, p. 73.
5) B.S. Nikol'skaya and A.I. Shreter, *Med. prom. S. S. S. R.*, **15**, No. 9, 47 (1961).
6) T. Inoue, C. Nakata, and K. Izawa, *Shoyaku*, **24**, 76 (1970).
7) N. Sakurai, T. Inoue, and M. Nagai, *Yakugaku Zasshi*, **92**, 724 (1972).
8) G. Illing, *Arzneim-. Forsch.*, **7**, 497 (1957).

spectrum of II showed signals (Table I) that allowed assignment as norvisnagin. Norvisnagin obtained by the demethylation[9,10] of visnagin was first isolated from natural source. III, $C_{15}H_{16}O_5$ and IV, $C_{13}H_{10}O_4$ were assigned as visamminol and visnagin by elemental analyses and spectral means (Table I), respectively.

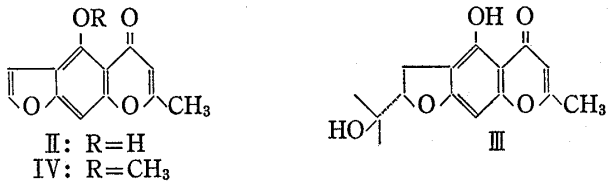

Chart 1

TABLE I

	=C–CH_3	Olefinic H	Ring H	Fran Ring H	Me
II	2.38	6.03	6.95	6.95 (overlap) 7.56 (d, J=2.5 Hz)	—
IV	2.32	6.04	7.25	7.04 (d, J=2.5 Hz) 7.60 (d, J=2.5 Hz)	4.15 (OCH_3)
III	2.32	6.00	6.28	dihydrofuran 3.11 (2H, d, J=9 Hz) 4.74 (1H, t, J=9 Hz)	1.22 1.33

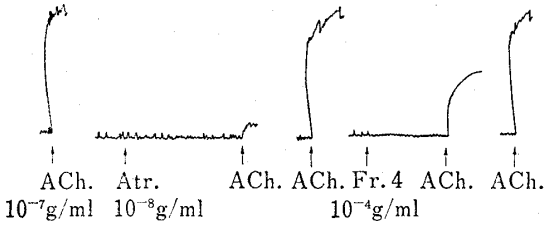

Fig. 1

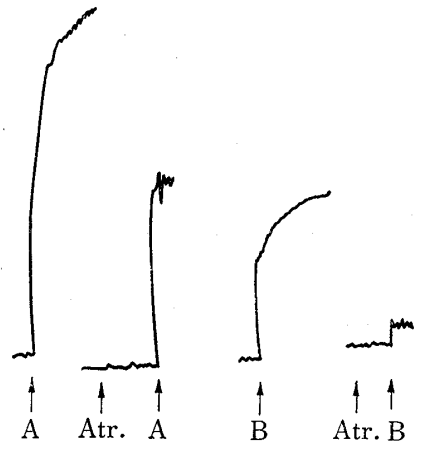

Fig. 2

A: Aq. Soln. 3×10^{-3} g/ml
B: Aq. Soln. 10^{-3} g/ml

As mentioned above, the extracts of the rhizoma of *C. dahurica* showed the spasmolytic effect. It was of interest, on the other hand, the residual aqueous solution had the spasmogenic effect on isolated strips of guinea pig jejunum (Fig. 2). Substances obtained from the chloroform fraction were tested the spasmolytic action. III at a dose of 5×10^{-5} g/ml antagonized to half the spasmodic effects of 10^{-7} g/ml acetylcholine chloride, 10^{-6} g/ml histamine chloride and 3×10^{-4} g/ml barium chloride (Fig. 3, 4 and 5). IV also showed spasmolytic activity

9) A. Schönberg and N. Badran, *J. Am, Chem. Soc.*, **73**, 2960 (1915).
10) A. Mustafa, M.M. Sidky, S.M.A.D. Zayed, and W.M. Abdo, *Mh. Chem.*, **98**, 310 (1967).

which was approximately one third as strong as III on isolated jejunum of guinea pig. However, the spasmolytic action of II was almost negligible.

Their activities compared with papaverine hydrochloride are shown in Table II.

Table II

Substances	Amounts required to neutralize effects of 3 mg $BaCl_2$ on guinea pig jejunum
I	inactive
II	inactive
III	1.5 mg
IV	4.5 mg
Papaverine HCl	0.52 mg

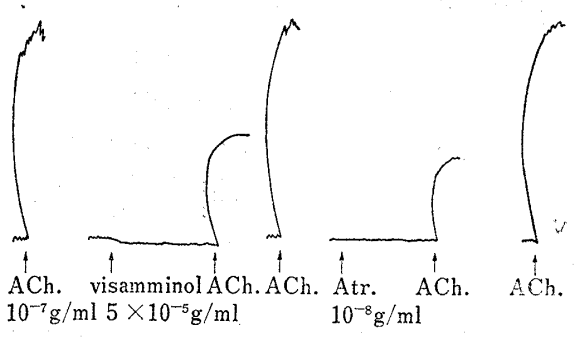

Fig. 3

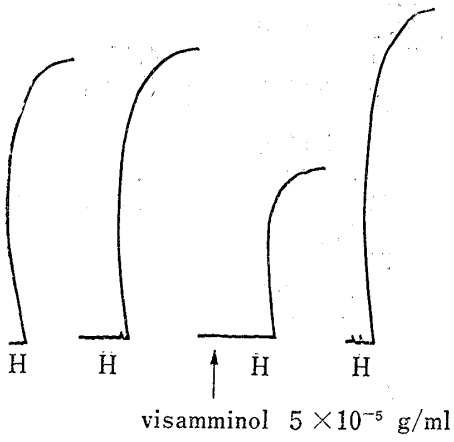

Fig. 4

H: Histamine–HCl 5×10^{-7} g/ml

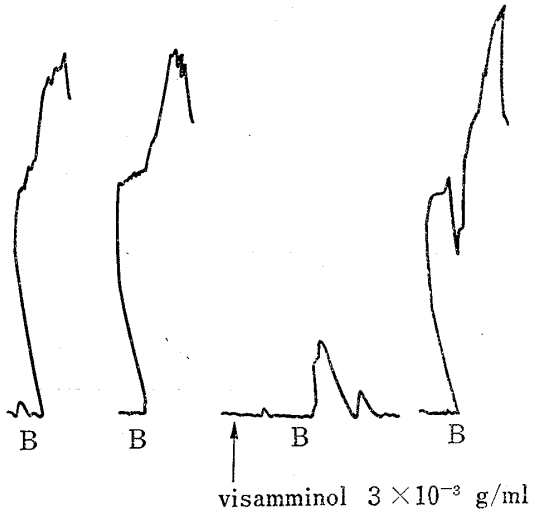

Fig. 5

B: $BaCl_2$ 3×10^{-4} g/ml

It is reported that a furochromone derivative, khellin, exhibits marked spasmolytic effect to the smooth muscles (urinary bladder, uterus and so on).[11]

We believe that the furochromones are principal constituents responsible for the efficacy of the Cimicifugae Rhizoma.

Experimental[12]

Isolation of Isoferulic Acid (I), Norvisnagin (II), Visamminol (III) and Visnagin (IV)——Air dried rhizoma of *Cimicifuga dahurica* Maxim. (Ranunculaceae) (52 kg) was extracted with 50% MeOH three times and the combined extract was concentrated until 10 liter *in vacuo*. The viscous aqueous solution was extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated under the reduced pressure to leave a brown viscous residue (*ca.* 1 kg) which showed five spots on TLC ($CHCl_3$–MeOH, 9: 1). 400 g of the $CHCl_3$ extract was subjected to chromatography on a silica gel column. The column was eluted progressively with $CHCl_3$ and a mixture of $CHCl_3$–MeOH. Each eluate was examined for spasmolytic activity (see pharmacological test).

11) K. Uhlenbroock and K. Mulli, *Arzneim.-Forsch.*, 7, 166 (1957).

12) All melting points were taken on a Yamato Model MP-21 apparatus and are uncorrected. IR spectra were recorded on a Shimazu IR-27G grating spectrophotometer, and UV spectra were taken on a Hitachi 124 spectrophotometer. NMR spectra were taken on a Hitachi R-20 spectrometer. The chemical shifts (δ) were calculated on the basis of TMS as an internal standard. Mass spectra were determined on a Hitachi RMU-7 mass spectrometer. Thin-layer chromatography was carried out on plates coated silica gel GF_{254} (Merk).

Fr. No.	ED_{50}[a]	Eluent	Fr. No.	ED_{50}[a]	Eluent
No. 1	10^{-3}		No. 9	inactive	
2	10^{-4}		10		
3			11		$CHCl_3$–MeOH (95:5)
4	3×10^{-5}	$CHCl_3$	12	10^{-3}	
5			13		
6	10^{-4}		14		
7			15	inactive	$CHCl_3$–MeOH (9:1)
8					

a) 10^{-7} g/ml of acetylcholine chloride

Fraction 14 gave isoferulic acid, mp 228° (from MeOH). Fraction 4 was rechromatographed on a silica gel column. The column was eluted with a mixture of pet–benzine–acetone (99: 1) to give a white solid which after recrystallization from MeOH gave norvisnagin (II) as yellow needles, mp 154.5—156.5° (lit.,[9] 156—158°; lit.,[10] 160°). *Anal.* Calcd. for $C_{12}H_8O_4$: C, 66.67; H, 3.73. Found: C, 66.64; H, 3.85. M^+ 216. IR (KBr) cm^{-1}: 2750, 1645, 1615, 1615, 1583. UV $\lambda_{max}^{95\%\ EtOH}$ nm (log ε): 214 (4.66), 246 (sh., 4.86), 253 (4.92), 261 (sh., 4.76), 282 (4.16), 336 (3.96); $\lambda_{max}^{95\%\ EtOH\ at\ pH\ 11}$ nm (log ε): 220 (4.66), 263 (sh., 4.81), 269 (4.82), 296 (4.13), 318 (4.18), 370 (3.99).

Subsequent eluate using mixtures of pet-benzine–acetone (98: 2—95: 5) gave a crystalline solid. Recrystallization from a mixture of pet-benzine–acetone gave (2S)-visamminol (III)[13] as colorless needles, mp 154—156° (lit.,[8] 160°). $[\alpha]_D$ +91.9° ($CHCl_3$, $c=0.2$). *Anal.* Calcd. for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84. Found: C, 65.28; H, 5.71. M^+ 276. IR (KBr) cm^{-1}: 3330, 3240, 1662, 1628, 1586.

Last fractions using mixtures of pet-benzine–acetone (9: 1—8: 2) as eluent gave a crystalline solid. Recrystallization from a mixture of pet-benzine–acetone gave visnagin (IV) as pale yellow needles, mp 141—143° (lit.,[8] 144°). *Anal.* Calcd. for $C_{13}H_{10}O_4$: C, 67.82; H, 4.38. Found: C, 67.80; H, 4.31. M^+ 230. IR (KBr) cm^{-1}: 1650, 1618, 1589.

Pharmacological Test——Guinea pigs used were male, Hartley strain (350—400 g). Spasmolytic effects were evaluated on isolated jejunum of guinea pig using acetylcholine chloride (10^{-7} g/ml), histamine hydrochloride (10^{-6} g/ml) and barium chloride (10^{-4} g/ml) as spasmogens.

Acknowledgement We are grateful to the members of the Central Analysis Room of this Institute for elemental analyses and measurement of NMR spectra.

13) Y. Kondo and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **20**, 1940 (1972).