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Pharmacological Studies on Pueraria Root. I. Fractional Extraction of Pueraria Root and Identification of Its Pharmacological Effects

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Pueraria root was extracted consecutively with acetone, methanol, and water, and 13 fractions (PA1-PA5, PM1-PM5, PW1-PW3) were prepared for basic pharmacological examinations. Many pharmacological actions were separated as fractionation of the extract progressed. Coexistence of substances having a mutually reverse pharmacological effect was found in the crude drug.

Acute toxicity ot each fraction was very weak. PA4, PA5, PM2, PW2, and PW3 decreased body temperature of mice, while PM4, PM5, and PW2 increased it. PA3, PA4, PA5, PM2, and PM4 exerted a papaverine-like action on the isolated guinea pig ileum, while PM1, PM3, and PM5 contracted this organ. PA5 and PM2 relaxed while PM3 contracted, the isolated guinea pig taenia coli. PA3, PA5, and PM2 showed a papaverine-like action on the isolated rat uterus. PA3, PA5, and all PM fractions contracted the isolated guinea pig vas deferens. PM2, PM3, and PG1 which was regarded as the component of PM2 potentiated the noradrenaline-induced contraction in this organ. PA3, PA5, all PM fractions, and PW2 inhibited electrically induced muscle contraction in the isolated frog sciatic nerve-sartorius muscle preparation. PA3, PA5, PM1, PM2, and PM4 contracted the isolated frog rectus abdominis muscle. PA3, PA5, PM1, PM3, PM4, PM5, and PW2 decreased blood pressure in anesthetized dogs, while PM2 elevated it. PA3, PA5, PM1, PM3, PM4, PM5, and PW2 increased femoral arterial blood flow in anesthetized dogs.

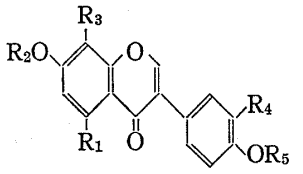
Some discussions were made on the pharmacological actions of each fraction.

Pueraria root (J.P. VIII) is prepared from the root of *Pueraria lobata* Ohwi and *Pueraria pseudo-hirsuta* Tang et Wang (Leguminosae), and is one of the most important crude drugs in Chinese medicine. "Koken tao" (in Chinese) or "Kakkon-to" (in Japanese) is a prescription in which pueraria root is prescribed as a chief ingredient (pueraria root 6.0 g, ephedra 4.5 g, ginger 3.0 g, jujube 2.5 g, Chinese cinnamon 3.5 g, Chinese peony root 3.5 g, licorice 2.0 g per day). Pueraria root has been considered to be effective for relaxing stiff shoulders and regarded as a diaphoretic, antipyretic, antitussive, and antidiarrheic.

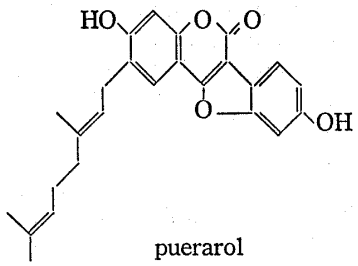
As specific components, many isoflavone derivatives, beginning with daidzein and puerarin, have been characterized by Shibata, *et al.*[2] as shown in Chart 1, and a cholinester-like substance was reported by Miura, *et al.*[3] Pharmacological effects of pueraria root reported so far include an antipyretic action of its extract by Tanno,[4] a papaverine-like musculotropic action of daidzein by Shibata, *et al.*,[5] and a cholinergic action of a cholinester-like substance by Miura, *et al.*[3]

In order to develop the study started by Shibata, *et al.*,[5] pueraria root was systematically extracted and basic pharmacological examinations were carried out on each fraction in the present study.

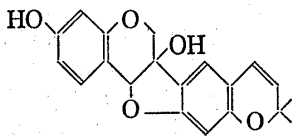
1) Location: *33–1, Yayoi, Chiba, 280, Japan.*
2) S. Shibata, T. Murakami, and Y. Nishikawa, *Yakugaku Zasshi*, **79**, 757 (1959); S. Shibata, T. Takizawa, and Y. Nishikawa, Abstr. Papers, 90th Annual Meeting of Pharmaceutical Society of Japan, II-206 (1970); S, Shibata and T. Takizawa, Abstr. Papers, 92nd Annual Meeting of Pharmaceutical. Society of Japan. II-225 (1972); S. Shibata and T. Takizawa, Abstr. Papers, 93rd Annual Meeting of Pharmaceutical Society of Japan. II-218 (1973).
3) K. Miura, T. Takeda, Y. Nakamoto, and H. Saito, *Oyo Yakuri*, **5**, 247 (1971).
4) Y. Tanno, *Nippon Yakurigaku Zasshi*, **33**, 263 (1941).
5) S. Shibata, M. Harada, and T. Murakami, *Yakuguku Zasshi*, **79**, 863 (1959).



	R_1	R_2	R_3	R_4	R_5
Formononetin	H	H	H	H	CH_3
Genistein	OH	H	H	H	H
Daidzein	H	H	H	H	H
Daidzin	H	glu.	H	H	H
Puerarin	H	H	glu.	H	H
PG–1	H	H	sugar	OH	H
PG–2	H	H	sugar	OCH_3	H



puerarol



kakkonein

Chart 1. Structural Formula of Isoflavone Derivatives and Other Compounds contained in Pueraria Root[2)]

Experimental

Crude Drug——Commercial pueraria root on the market (Japanese origin) was used.

Method of Fractional Extraction——Fractional extraction of the crude crug was conducted according to the procedure shown in Chart 2.

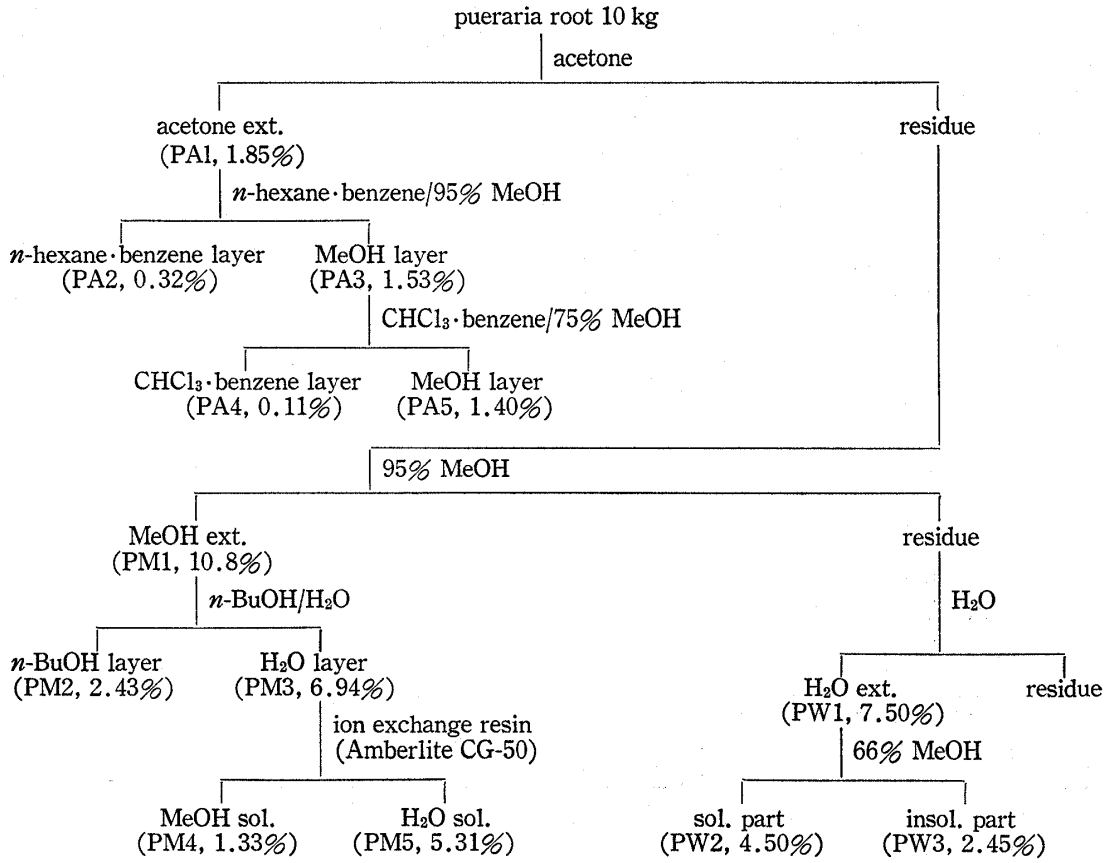


Chart 2. Fractional Extraction of Pueraria Root

Materials for Pharmacological Examination——All dried fractions separated by the above procedure were used and acetone- and methanol-extracted fractions were mainly examined. PG1, which was regarded as the main component of PM2 fraction, was also used in one of the tests. Each material was dissolved or suspended in physiological saline solution, adjusted to pH 7 with NaOH if necessary.

Animals——Male dd strain mice (18—24 g), female Wistar strain rats (180—220 g), male guinea pigs (300—450 g), mongrel dogs of either sex (6—10 kg), and frogs of either sex (25—35 g) were used.

Pharmacological Test Methods——1) Acute Toxicity: Acute toxicity was examined in mice for a period of 48 hr. Materials were administered intraperitoneally (*i.p.*).

2) Effect on Body Temperature in Mice: After injection of the test material (1 g/kg, *i.p.*), rectal temperature of the mouse was measured every 30 min for 4—6 hr. During the experimental period, animals were kept in individual cages maintained at an ambient temperature of 22—24°.

3) Effect on the Isolated Organs (organ bath method, isotonic recording on a kymograph): (a) Effect on guinea pig ileum: Tyrode solution (NaCl 8.0 g, KCl 0.2 g, $CaCl_2$ 0.2 g, $MgCl_2$ 0.1 g, NaH_2PO_4 0.05 g, $NaHCO_3$ 1.0 g, glucose anhydr. 1.0 g, distilled water added to make 1000 ml) kept at 28° was used as a bath fluid. Four to 6 preparations were employed for each dose. (b) Effect on guinea pig taenia coli: Tyrode solution kept at 31° was used. Three to 5 preparations were employed for each dose. (c) Effect on rat uterus: Rats were administered 0.1 mg/kg of diethylstilbestrol, subcutaneously 18—21 hr before sacrifice. De Jalon solution (NaCl 9.0 g, KCl 0.42 g, $CaCl_2$ 0.06 g, $MgCl_2$ 0.005 g, $NaHCO_3$ 0.5 g, glucose anhydr. 0.5 g, distilled water added to make 1000 ml) kept at 28° was used as a bath fluid. Five to 6 preparations were employed for each dose. (d) Effect on guinea pig vas deferens: Tyrode solution kept at 31° and bubbled with O_2 was used. The organ was allowed to stand in the bath for at least 1 hr before the test. Four to 8 preparations were employed for each dose. (e) Effect on frog sciatic nerve-sartorius muscle preparation: The preparation was set in frog Ringer solution (NaCl 6.0 g, KCl 0.075 g, $CaCl_2$ 0.1 g, $NaHCO_3$ 0.1 g, distilled water added to make 1000 ml) bubbled with O_2. The nerve was stimulated with a square wave pulse either supramaximally or submaximally (0.1 Hz, 0.2-msec duration). Twitches of the muscle were isometrically recorded using a force displacement transducer (Nihon Kohden, SB-1T). Three to 6 preparations were employed for each dose. (f) Effect on frog rectus abdominis muscle: Frog Ringer solution was used as a bath fluid. Four to 6 preparations were employed for each dose. Through all the experiments made on isolated organs, wherever examinations for the interaction were conducted between the extracts and standard drugs, an agonistic substance was applied 3—5 min after the pre-application of another substance.

4) Effect on Blood Pressure in Dogs: Dogs were anesthetized with 40 mg/kg of pentobarbital sodium (*i.p.*) and tracheal intubation was made. Blood pressure was taken from the carotid artery by means of a mercury manometer. Drugs were injected *via* the femoral vein. Two to 6 animals were employed for each fraction.

5) Effect on Femoral Blood Flow in Dogs: Dogs were prepared for the test as above. Blood pressure was recorded through a pressure transducer (Nihon Kohden, MPU-0.5). After the animal was heparinized (300 units/kg, followed by 100 units/kg every 1 hr), vascular beds branching off from one femoral artery were autoperfused by the same artery *via* a polyethylene loop which passed through an electric flowmeter (Nihon Kohden, MF-2). The blood flow was recorded on a pen-writing recorder (Nihon Kohden, W1-130) together with blood pressure. Drugs were injected intra-arterially in a volume of 0.4 ml for 10 sec. Two animals were employed for each fraction.

Drugs——Standard drugs used were as follows: Acetylcholine chloride, aminopyrine, atropine sulfate, diphenhydramine hydrochloride, isoproterenol hydrochloride, histamine dihydrochloride, noradrenaline hydrochloride, papaverine hydrochloride, propranolol hydrochloride, tolazoline hydrochloride, and *d*-tubocurarine chloride. Doses refer to those of the salt.

Results

Fractional Extraction

Fractions were named PA1, PA2, PA3, PA4, PA5, PM1, PM2, PM3, PM4, PM5, PW1, PW2, and PW3. The yield of each fraction is given in Chart 2.

Pharmacological Effect

1) Acute Toxicity: PA4 was fatal to the mouse in a dose of 3 g/kg but not in 1 g/kg. In all other fractions, however, no death occurred even in a dose of 3 g/kg. In general, no marked behavioral change was observed in a dose of 0.3 g/kg of all fractions, while decrease of spontaneous movement, palpebral ptosis, abnormal gait, and writhing symptom developed with increasing doses.

2) Effect on Body Temperature in Mice: The result is given in Fig. 1. PA4, PA5, PM2, PW2, and PW3 decreased body temperature of mice. Among them, the effect of PA4 and PW3 was sustained and that of others was temporary. In contrast, PM4 and PM5 increased the body temperature at a late stage and PW2 also showed such an effect to a lesser extent. The hypothermic activity of PA4 and PW3 was the most marked and hyperthermic activity of PM5 was more potent than that of PM4.

3) Effect on Isolated Organs: (a) Effect on Guinea Pig Ileum: PA3, PA4, PA5, PM2, and PM4 had a weak papaverine-like action in concentrations of 5×10^{-4} to 3×10^{-3} g/ml,

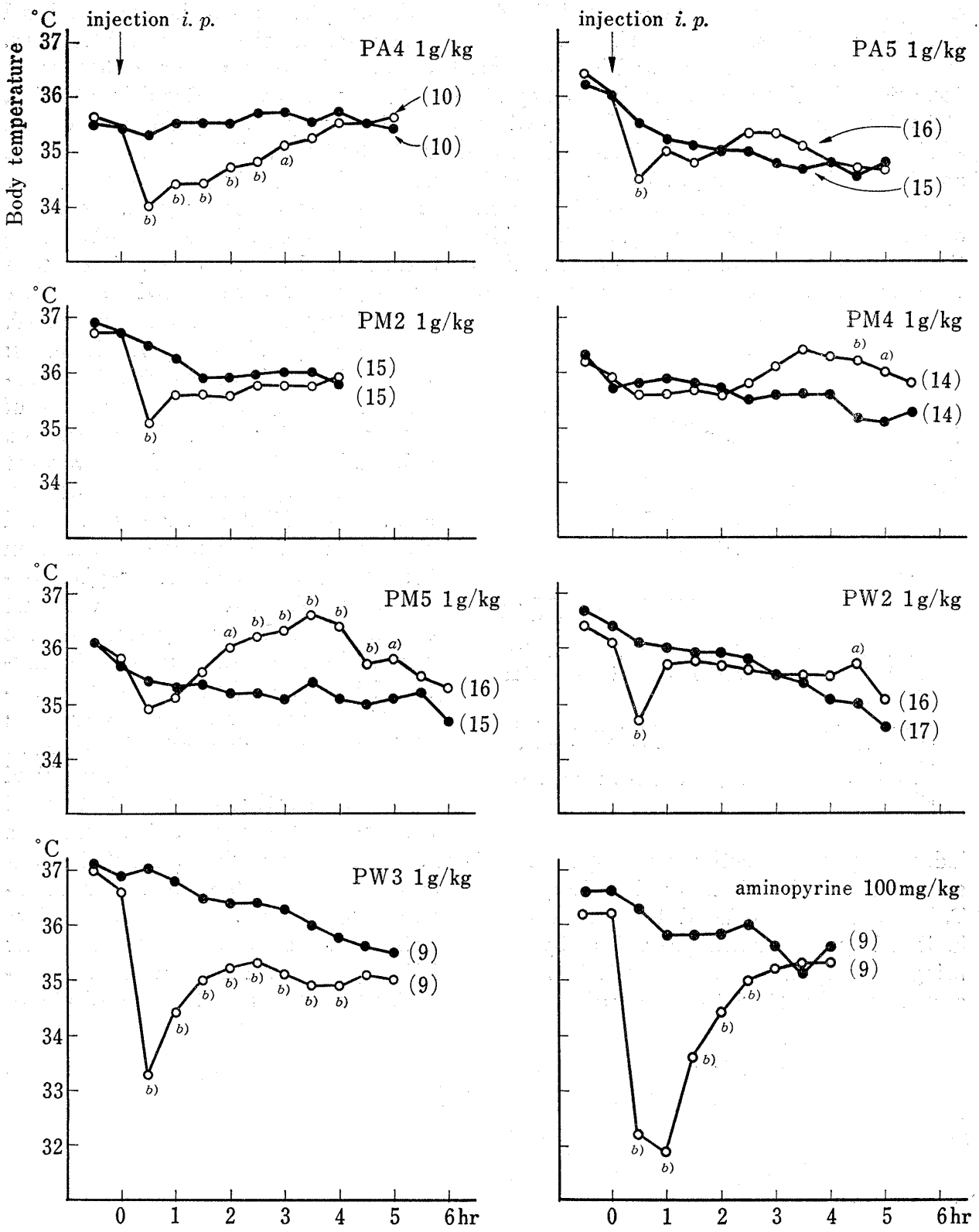


Fig. 1. Effect of the Fractions on Body Temperature in Mice

●——● :control, ○——○ :test : *a*) $p<0.05$, *b*) $p<0.01$
PA4 was dissolved in 2% Tween 80 saline solution.
Number in parentheses indicates number of animals.

displaying a non-competitive antagonistic effect to the acetylcholine- or histamine-induced contraction. The effect induced by PA fractions was more marked than that of PM fractions. On the contrary, PM1, PM3, and PM5 contracted the ileum in concentrations of 3×10^{-3} to 1×10^{-2} g/ml. The contraction induced by PM5, at 1×10^{-2} g/ml was reduced by atropine, at 5×10^{-9} g/ml and slightly by diphenhydramine, at 1×10^{-10} g/ml. (b) Effect on Guinea Pig Taenia Coli: PA5 and PM2 relaxed the organ at 3×10^{-3} g/ml. This relaxation was not antagonized by tolazoline, at 1×10^{-4} g/ml or by propranolol, at 1×10^{-5} g/ml. On the other hand, PM3 contracted the taenia coli in concentrations of 3×10^{-3} to 1×10^{-2} g/ml. (c) Effect on Rat Uterus: As shown in Fig. 2, PA3 and PA5 inhibited the acetylcholine-induced contraction at 3×10^{-3} g/ml, while PM2 was less effective and PM3 had no such effect. These tests were carried out by a cumulative method. No contracting action was observed up to a dose of 1×10^{-2} g/ml of all fractions tested. (d) Effect on Guinea Pig Vas Deferens: PA3, PA5, and all PM fractions contracted the organ in concentrations of 1×10^{-3} to 1×10^{-2} g/ml. The contraction induced by PA5, PM2, and PM3 was not antagonized by tolazoline, at 3×10^{-5} g/ml. PM2, PM3, and PG1 potentiated the noradrenaline-induced contraction in 5×10^{-4}, 2×10^{-3}, and 1×10^{-4} g/ml, respectively. These doses alone were not capable of producing any effect on the organ. This potentiating effect is illustrated in Fig. 3. (e) Effect on Frog Sciatic Nerve-Sartorius Muscle Preparation: Under a supramaximal stimulation, PA3, PA5, all PM fractions, and PW2 inhibited muscle contraction in concentrations of 3×10^{-3} to 1×10^{-2} g/ml. The inhibition induced by each fraction was more strengthened when a submaximal stimulation was employed, ranging from an inhibitory rate of about 50% to 100% in 2—5 min. *d*-Tubocurarine also reduced the muscle contraction at 1×10^{-5} g/ml. Muscle contractions inhibited by each fraction as well as by *d*-tubocurarine returned to the original level after being washed with the nutrious solution. PW1 and PW3 potentiated the contraction in concentrations of 5×10^{-3} to 1×10^{-2} g/ml. Ash from both fractions, however, showed almost

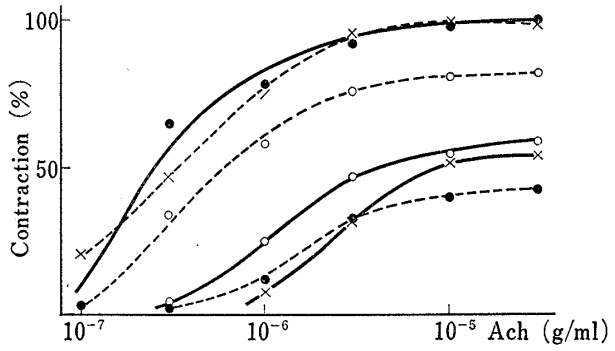


Fig. 2. Effect of the Fractions on Acetylcholine (Ach)-induced Contraction of the Isolated Rat Uterus

●——●:control ○——○:PA5 3×10^{-3} ×------×:PM3 3×10^{-3}
●------● : PA3 3×10^{-3} ○------○ : PM2 3×10^{-3} ×——× :papaverine 1×10^{-5} (g/ml)
The number of experimental preparations was 5—6 for each point.

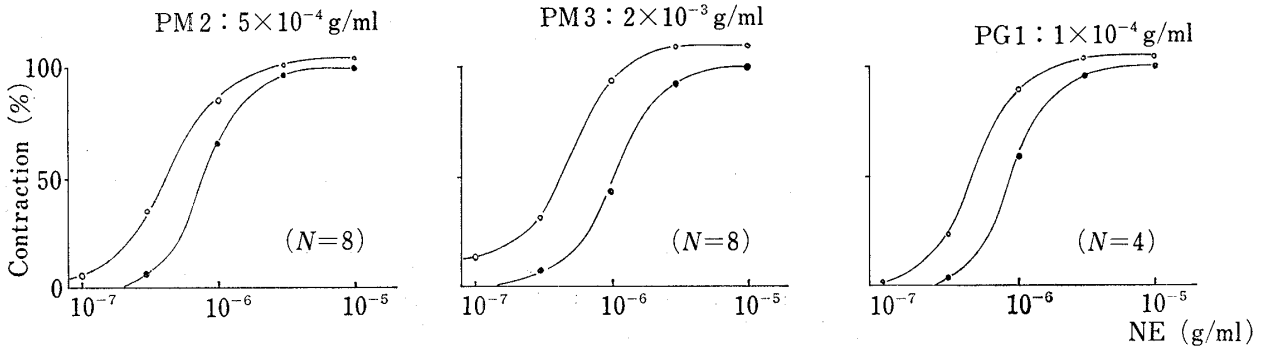


Fig. 3. Effect of PM2, PM3, and PG1, on Noradrenaline(NE)-induced Contraction of the Isolated Guinea Pig Vas Deferens

●——● : control ○——○ : PM2, PM3 or PG1-pretreated preparation
N indicates the number of experimental preparations.

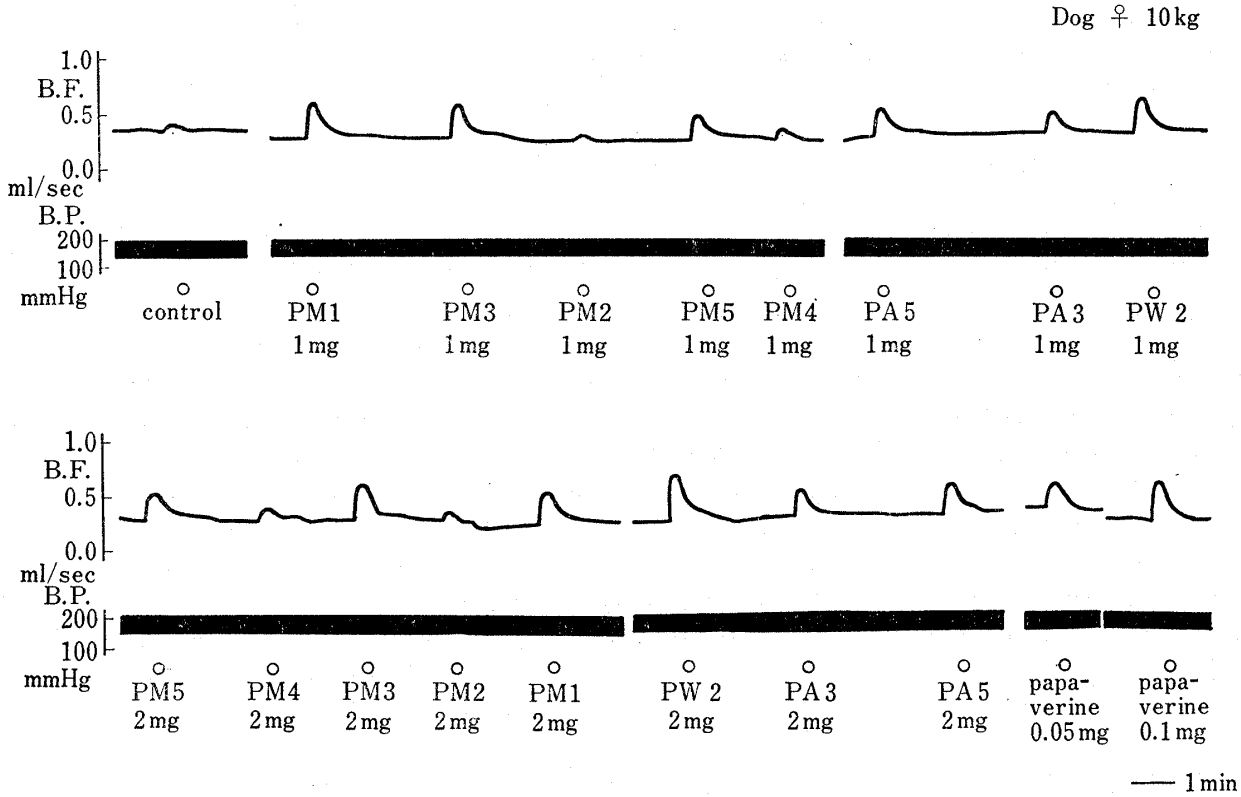
the same action as did the extracts in an amount equal to that contained in the original solution. On the contrary, PW2 lost its effect after ashing. (f) Effect on Frog Rectus Abdominis Muscle: The organ was contracted by PA3, PA5, PM2, PM1, and PM4 in 1×10^{-2} g/ml, and the potency of each fraction decreased in that order. PM3 and PM5 were slightly or not effective. The contraction induced by PA3 and PM2 was nearly comparable with those elicited by acetylcholine, at 5×10^{-6}, and 1×10^{-6} g/ml, respectively. These contractions induced by the extracts were not antagonized by *d*-tubocurarine, at 1×10^{-6} g/ml.

4) Effect on Blood Pressure in Dogs: The result is given in Table I. PM2 alone showed a temporary elevation in blood pressure in a dose of 15 mg/kg and this elevation was followed by a fall when the dose was increased. All other fractions induced fall in blood pressure at 30

Table I. Effect of the Fractions on Blood Pressure in Anesthetized Dogs

Fraction	Dose (*i.v.*) (mg/kg)	No. of animals	Blood pressure (mmHg)	
			decrease	increase
PA3	30	2	20—25	
PA5	30	3	10—25	
PM1	30	3	30	
PM2	15	4		10
PM3	30	6	20—50	
PM4	30	4	30—50	
PM5	30	3	0—10	
PW2	30	3	30—50	

initial blood pressure: 130—160 mmHg



Intra-arterial injection was employed.

Fig. 4. Effect of the Fractions on Femoral Blood Flow (B.F.) and Blood Pressure (B.P.) in an Anesthetized Dog

mg/kg, which recovered to the original level in 3 min. The fall induced by PA3 and PA5 was not protected by 1 mg/kg of atropine, 2 mg/kg of diphenhydramine, or 1 mg/kg of propranolol. The fall induced by PM5 was antagonized by atropine, 1 mg/kg.

5) Effect on Femoral Flow in Dogs: A typical recording is presented in Fig. 4. PA3, PA5, PM1, PM3, PM4, PM5, and PW2 increased the femoral blood flow. The potency of these materials was about 1/10 to 1/20 of papaverine. On the other hand, PM2 did not give such an effect and PM4 was slightly effective.

Discussion

Preliminary examination by thin-layer chromatography of each fraction showed that, qualitatively, isoflavones are present in the lipophilic fractions (PA's and PM2), but not in the hydrophilic fractions (PM3, PM4, and PM5), and isoflavone glycosides are present in both fractions. As the fractionation progressed, pharmacological effect was separated into several fractions. Considerable pharmacological difference was found in the lipophilic fractions and hydrophilic fractions as mentioned in the Results.

Acute toxicity of each fraction was very weak. The action of the extract on body temperature in mice became different as its fractionation progressed. Hypothermic activity appeared in the lipophilic fractions and PW fractions, and hyperthermic activity in the hydrophilic fractions. The vasodilatation induced by the lipophilic fractions and PW2 discussed below seems to contribute to their hypothermic effect. In the case of the hydrophilic fractions, however, it is inferred that some calorigenic effect surpasses heat loss possibly originating from their vasodilating effect. As pueraria root is a natural product, there remains the possibility that some pyrogen-like substance(s) might be present in the crude drug. However, PM5, which showed the most marked hyperthermic activity, was prepared from the methanol-extracted fraction and it lost this activity after dialysis (unpublished data). Accordingly, it seems that such a possibility might be slight, if at all. These facts suggest the presence of a specific substance in pueraria root responsible for such a hypothermic or hyperthermic effect.

In the isolated guinea pig ileum, the lipophilic fractions and PM4 had a nonspecific antispasmodic effect. On the contrary, contraction of the organ was observed in PM1 and this activity shifted to PM5. The action of PM5 appeared to be mainly cholinergic, which may be identical with that reported by Shibata, *et al.*[5] and by Miura, *et al.*[3] The relaxing action of PA5 and PM2 on the isolated guinea pig taenia coli was not adrenergic and was assumed to depend on their nonspecific antispasmodic property. PM3 seems to affect the organ mainly through a cholinergic activity. In the isolated rat uterus, the lipophilic fractions again showed a nonspecific antispasmodic action. Through all the tests made on these isolated smooth muscle organs, antispasmodic activity was not found in any of the fractions, the potency of which went up to 1% of that of papaverine. It was demonstrated in the isolated mouse ileum that antispasmodic activity of daidzein, which is mainly distributed in PA4, was about 1/3 that of papaverine, whereas daidzin or puerarin, a glucoside of daidzein, lost such an effect to a great extent.[5] Since the antispasmodic potency of PA fractions was better than that of PM fractions, isoflavone compounds like daidzein may be the most responsible principle for this activity. In the isolated guinea pig vas deferens, contracting action of PA3 and PA5 was not adrenergic and that of PM5 was thought mainly cholinergic. The potentiating action shown by PM2 and PM3 of noradrenaline-induced contraction in the vas deferens preparation may partly be ascribed to that induced by PG1, an isoflavone glycoside. In the test performed on the frog nerve-muscle preparation, it was found that inorganic substances present in the extract markedly influenced the pharmacological action. Therefore, it is difficult to say that the potentiating effect of muscle contraction induced by PW1 and PW3 was inherent in pueraria

root. The contracting action of the lipophilic fractions on frog rectus abdominis muscle was not cholinergic.

The increase of femoral blood flow in dogs elicited by the lipophilic fractions may be due mainly to its antispasmodic property affecting vascular beds, and that produced by PM5 may chiefly depend on its cholinergic activity. It appears that vasodilating effect of almost all the fractions plays a considerable part for the blood pressure lowering action of respective fractions. PM2 was characteristic in that it did not increase femoral blood flow and elevated blood pressure.

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