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Pharmacologically Active Principle of *Piper retrofractum*

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필발의 藥物活性成分

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A pharmacologically active constituent was separated from the methanol extract of the seeds of *Piper retrofractum* and identified as piperine.

Introduction

The fruits of various *Piper* species have long been used as a condiment and a medicinal in the treatment of various abdominal or intestinal disorders in oriental medicines and as folkloric remedies[1~3].

Although numerous phytochemical and pharmacological studies have been reported concerning various *Piper* species[4~11], a few investigations on the constituents of *Piper retrofractum* have been reported[12~14].

The methanolic extract of the fruits of this plant was recently found to show a potentiating activity on hexobarbital induced sleeping time and anti-strychnine toxic activity as well as inductive effects on drug metabolizing enzyme activity[15]. This paper describes the isolation of an active compound, piperine.

In order to isolate the active principle, the water-insoluble fraction of the methanol extract of the fruits was chromatographed on Silica gel using the solvent systems of benzene : ether (4:1) and benzene : ether : methanol(4:1:1).

The various fractions obtained were subjected to examination for the potentiating activity of sleeping time in mice.

As the results, it was found that the fraction numbers 10 and 11 exhibited most potent activity and gave a yellow spot (Rf 0.19 and Rf 0.72) which was detected by spraying 50% sulfuric acid on thin layer plate (Table I).

From the active fractions, piperine was isolated and identified by the comparison of its mp, NMR and MS spectral data with reported ones.

Pharmacology

Piperine was assessed for CNS-depressant activity by general behavior test and two other different methods; 1) potentiating effect on hexobarbital hypnosis, 2) effect on strychnine mortality in mice.

The results of the two tests are indicated in Table II. Piperine, at the dose of 1/10 of its LD_{50} value, showed a strong potentiating effect on hexobarbital induced hypnosis(122.4% increase in sleeping time). It was also noted that piperine exerted a potent anticonvulsant effect as measured by protection against strychnine mortality.

In general behavior evaluations, piperine, at

Table I. Chromatographic Fractions of Methanolic Extract of *Piperis retrofracti* Fructus and their Effects on Hexobarbital Hypnosis.

Fraction (No.)	Weight (g)	Rf values of spots	Potentiation of Hexobarbital hypnosis[c] (% increase)
1	0.04	0.82	16.0(N. S.)[d]
2	1.15	0.82 0.74	8.9(N. S.)
3	3.22	0.74 0.62	160.9($p<0.01$)
4	0.30	0.92 0.53	127.6($p<0.01$)
5	0.90	0.45	97.7($p<0.01$)
6	4.60	0.45 0.36	123.0($p<0.01$)
7	2.64	0.36 0.30	179.3($p<o.01$)
8	3.25	0.30	305.6($p<0.001$)
9	2.90	0.24 (0.09)[a]	132.8($p<0.01$)
10	27.78	(0.24) 0.19[b] (0.09)	571.7($p<0.0001$)
11	1.89	0.72[b] (0.61) (0.51)	518.5($p<0.0001$)
12	1.76	(0.72) 0.61 (0.51)	107.8($p<0.01$)
13	8.16	(0.61) 0.51	69.2($p<0.01$)
14	1.18	0.46	39.3($p<0.05$)
15	1.40	0.38	13.1(N. S.)
16	2.64	0.07	41.1($p<0.05$)

Solvent systems : benzene : ether (4:1) [Fraction No. 1-10] and benzene : ether : methanol (4:1:1) [Fraction No. 11-16]; spraying reagent; 50% H_2SO_4

a) Rf values in parentheses indicate ones for minor spots.

b) The substance with Rf value of 0.19 in fraction No. 10 was identical to the one with Rf value of 0.72 in fraction No. 11.

c) Mice were treated i.p. with 100 mg/kg of each fraction 30 min prior to the injection of hexobarbital sodium (50 mg/kg i.p.). Six mice were used far each group.

d) Figures in parentheses indicate results of student t-test. N.S., not significant.

doses of 30 and 50 mg/kg i.p., exhibited significant decrease in spontaneous movement, and increase in passivity and ptotic symptoms and lowering in rectal temperature (2°C).

Table II. Pharmacological Properties of Piperine.

Compounds	Potentiation of hexobarbital sleeping time (min)[a]	Strychnine mortality in 30 min (survived/used)
Control	15.2±1.1	2/10
Piperine (30 mg/kg, i.p.)	33.8±1.9[b]	10/10

a) Data were expressed as means± S.E.M. Six mice were used for each group.

b) Significantly different from control, $p<0.001$.

Experimental

The fruits of *Piper retrofractum* was obtained commercially in Seoul and was identified by Dr. Hyung Joon Chi of this institute. Male dd mice weighing 20±2 g were used in animal tests.

Isolation of piperine The fruits (2.8kg) were crushed, refluxed with methanol for 8 hrs, and filtered. This procedure was repeated three times and the combined filtrate was concentrated under the reduced pressure and suspended in water. The water-insoluble material was collected by filtration, washed with

water and dried. This water-insoluble matter (65g) was chromatographed on Silica gel and eluted with benzene : ether : MeOH (4:1:1). 31.2 g of crude piperine was separated as a major component (Table I) from fraction numbers 10 and 11.

The crude piperine separated was purified by recrystallization from ethanol several times.; mp., 130°; λ_{max} nm (logε): 224(4.04), 302 (4.22), 310(4.27), 345(4.46); IRν_{max}: 1630, 1610cm^{-1}(acid amide) 1580, 1000cm^{-1} (*trans* conjugated double bond) 1250, 1025, 930cm^{-1} (-O-CH_2-O-); NMR(100 MHz, $CDCl_3$)δ: 1.64 (6H, m, cyclic), 3.6(4H, m, cyclic), 5.98 (2H s, -O-CH_2-O-), 6.36-7.55 (7H, m, aromatic and olefinic); MS, m/e (refl. int.): 285(M,$^+$ 32.5), 201(M$^+$- C_5H_{10} N, 63), 173 (M$^+$- C_6H_{10} ON, 30.5), 143 (173-CH_2O, 31.5), 115(143 - CO, 100), 84($C_6H_{10}ON$, 53).

The physico-spectral data were in agreement with published data[16,17].

Determination of hexobarbital induced hypnosis[18] Indicated amounts of the materials suspended in 0.5% carboxymethyl cellulose solution were administered intraperitoneally. The control group was treated with vehicle only. Hexobarbital sodium (50 mg/kg, i.p.) was administered 30 min after the administration of the material and the duration of sleeping time was determined.

Determination of strychnine mortality[19] The material was injected intraperitoneally 30 min prior to the administration of strychnine nitrate (1.2 mg/kg, i.p.). This dose of strychnine nitrate caused a tonic convulsion and more than 50% mortality within 30 min in untreated control mice. The mice were observed for 30 min and the mortality was recorded.

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