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Isolation of Dillapiol from a Chemotype of *Perilla frutescens* as an Active Principle for Prolonging Hexobarbital-Induced Sleep

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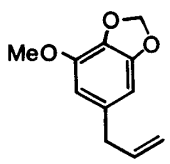
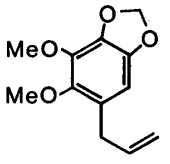
The effects of MeOH extracts from six distinct chemotypes of *Perilla frutescens*, which differ in the main components of the essential oils, on the prolongation of hexobarbital-induced sleep in mice were examined. Dillapiol, 1-allyl-2,3-dimethoxyl-4,5-(methylenedioxy)-benzene, was isolated from the MeOH extract of the chemotype PP-DM as an active principle (ED_{50} = 1.57 mg/kg).

Keywords——*Perilla frutescens*; Labiatae; sleeping time; chemotype; dillapiol

The dried leaf of *Perilla frutescens* BRITTON var. *acuta* KUDO (Labiatae) has been used as a sedative and an antipyretic in traditional Chinese medicine. Woo and Shin[1] reported that administration of the 70% MeOH extract (125 mg/kg, i.p.) of an unidentified chemotype of *Perilla* to mice caused a prolongation of sleeping time (358%), but the active principle was not identified. Of six genetically determined chemotypes of this species that differ in the main components of the essential oils,[2-5] only one chemotype (PA) containing perillaldehyde has been investigated for sedative activity, and perillaldehyde and stigmasterol were identified as the synergistic active principles.[6] We now report the effects of leaf extracts from six chemotypes of *Perilla* on hexobarbital-induced sleep in mice, and the isolation of dillapiol from a phenylpropanoid type (PP-DM), which contains dillapiol and myristicin (Chart 1) in the volatile oils,[7] as a highly active principle.



myristicin



dillapiol

Chart 1

Experimental

Plant Material——The six chemotypes of *Perilla frutescens* tested were cultivated in the same field in Kyoto and the leaves were harvested at the end of August. Dried leaves (100 g) of each chemotype were extracted with MeOH (1.4 l) at room temperature for two weeks, then the solvent was evaporated off under reduced pressure at 40 °C. The air-dried extract was suspended in 1% carboxymethylcellulose (CMC) in distilled water or dissolved in olive oil and administered to test animals at a dose of 10 ml/kg body wt. In all the experiments, CMC (1%) or olive oil was used as the control substance. For isolation of the active principle from PP-DM type, fresh leaves (800 g) were extracted with MeOH (9 l) at room temperature for 2 weeks. The extract was dried over $CaCl_2$ for 10 d after evaporation of the solvent.

Pharmacological Testing——Sleeping time was measured according to the method described elsewhere.[6] ED_{50} was calculated by Lichfield and Wilcoxon's method.

Gas Liquid Chromatography (GLC) and Proton Nuclear Magnetic Resonance (^{1}H-NMR) Analyses of Essential Oils——Fresh leaves were extracted with ether (1.25 ml/g fresh wt.) overnight at 4 °C. GLC analysis was carried out

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on a Hitachi 163 gas chromatogram using a stainless steel column (3 mm × 2 m) packed with 17% PEG-6000 in Chromosorb W (30—60 mesh) at a column temperature of 170 °C, with N_2 as the carrier gas at a flow rate of 30 ml/min, and FID as the detector.[4,7] ^{1}H-NMR was recorded on a JEOL JNM-PMX 60 spectrometer in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard.

Results and Discussion

Table I shows the results obtained from oral administration of the leaf extracts (2 g/kg) of six chemotypes (PA, L-PA, PK, EK, PP-M, and PP-DM) containing, as the main component, perillaldehyde, *l*-limonene, perillaketone, elsholtziaketone, myristicin, and dillapiol, respectively. All of these methanolic extracts significantly prolonged hexobarbital-induced sleep in mice; the extract of PP-DM type showed a higher activity than the other chemotypes.

When the leaf extract (7.3 g) of PP-DM type was distributed between $CHCl_3$ (2 l) and water (0.5 l), all the activity moved into the $CHCl_3$ extract (2.6 g). A part of the $CHCl_3$ extract (2.0 g) was chromatographed on a silica gel column (Wakogel C-200, 150 g), using $CHCl_3$ as the developing solvent to give three fractions; F 1 (0.21 g), F 2 (0.47 g), and F 3 (1.29 g). The main activity was found in F 2, which was then subjected to preparative thin layer chromatography (TLC) (silica gel, *n*-hexane–acetone, 19 : 1) to yield an oily substance (267 mg) showing a high activity (relative activity = 659% at 142 mg/kg).

Table I. Effects of Orally Administered MeOH Extracts of Six Chemotypes of *Perilla* on Hexobarbital-Induced Sleep in Mice

Extract administered	Dose (g/kg)	No. of mice	Sleeping time (min)	
			Mean ± S.E.	Relative activity
Control (1% CMC)	—	16	20.2 ± 2.1	100
PA	2.0	7	37.2 ± 3.4[c]	184
L-PA	2.0	7	30.7 ± 3.5[a]	152
PK	2.0	9	38.0 ± 5.5[b]	188
EK	2.0	6	38.0 ± 4.8[c]	188
PP-M	2.0	8	54.5 ± 9.4[c]	270
PP-DM	2.0	8	97.2 ± 6.3[c]	481

a) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$. PA, perillaldehyde type; L-PA, limonene-perillaldehyde type; PK, perillaketone type; EK, elsholtziaketone type; PP-M, myristicin type; PP-DM, dillapiol-myristicin type.

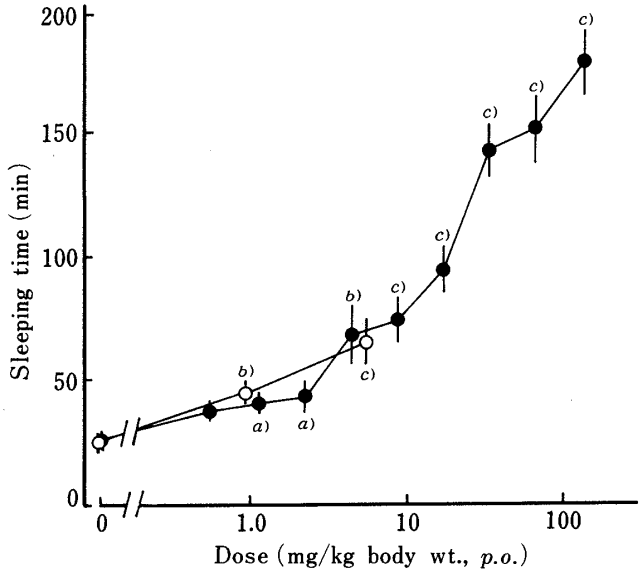

Fig. 1. Effect of Dillapiol and Chlorpromazine·HCL on Hexobarbital-Induced Sleep in Mice

Each point represents the mean ± S.E. Significantly different from each control group: *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$. ●—●, Dillapiol; ○—○, chlorpromazine·HCl.

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This substance was identified as dillapiol by GLC and ^{1}H-NMR.[8] $t_R = 5.9$ relative to perillaldehyde; δ ($CDCl_3$) = 3.23 (2H, d, $J = 6$ Hz, $-C\underline{H}_2-CH=CH_2$, 3.68 (3H, s, $-OC\underline{H}_3$), 3.93 (3H, s, $-OC\underline{H}_3$), 4.80, 5.05 (1H × 2, m, $-CH=C\underline{H}_2$), 5.70 (1H, m, $-C\underline{H}=CH_2$), 5.71 (2H, s, $-O-C\underline{H}_2-O-$), 6.22 (1H, s, aromatic proton). Its ED_{50} was estimated to be 1.57 mg/kg ($p < 0.05$). It is known that fresh leaves of PP-DM type contain, in addition to dillapiol (0.22—0.34% of fresh wt.), a small amount of myristicin (0.03—0.05%).[7] Although myristicin was reported to have a sleep-prolonging activity by Seto and Keup,[9] it was not isolated as an active substance in the present study. Accordingly, the activity of the leaf extract of PP-DM type was mainly due to dillapiol.

As shown in Fig. 1, dillapiol prolonged hexobarbital-induced sleep dose-dependently within the tested range, causing a statistically significant effect at any dose higher than 1.1 mg/kg (*p.o*). At the dosage levels less than 10 mg/kg, dillapiol was almost equivalent in activity to chlorpromazine. It should be noted, however, that continual convulsions were observed in many of the sleeping mice that had taken a high dose (> 18 mg/kg) of dillapiol, although none of the mice died within 72 h after administration, even at a dose of 500 mg/kg.

The extracts of PP-M and PP-DM types showed a higher activity on hexobarbital-induced sleep than that of the PA type, which is considered to be the best type in traditional Chinese medicine.[10] However, taking crude drugs of the former types in large quantities may cause problems, since they contain myristicin, known to be a hallucinogen.[11,12] It is possible that dillapiol, and analogue of myristicin, might also cause hallucination at a high dose.

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