Communications to the Editor

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AN ACTIVE COMPONENT OF PINE LEAVES EXHIBITING ANTI-SEROTONERGIC ACTION

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In examining the pharmacological effects of homeopathic medicines and other plant extracts on the contractile responses to serotonin in isolated rat aorta, the hexane:acetone (1:1) extract of pine leaves was found to possess an anti-serotonergic effect. The extract was further fractionated by column chromatography to identify the active constituents. The results showed that polyprenol is the active component in pine leaves exhibiting anti-serotonergic action.

KEYWORDS —— natural product; pine leave; polyprenol; anti-serotonergic effect; isolated rat aorta

Japanese red (Pinus densiflora Sieb. et Zucc.) and Japanese black pine (P. thumbergii Parl) are representatives of the pine family in Japan. There are, however, 10 families and 250 known species of pine in the temperate and semitropical zones of the northern hemisphere, such as tall evergreen trees and, though rare, smaller deciduous trees.[1] In medicinal usage, rosins obtained from plant secretions of the pinus family, from which volatile oils have been removed, are used as adhesives for medical plasters. Terebine oil, which is a volatile oil obtained from the trunk or balsam of the plants by distillation with water vapor, is used externally as a skin stimulant and rubefacient for rheumatic neuralgia and scabies. In China, the pine needles (pine leaves) of Chinese White pine (Pinus armandii Fronch) have been used as homeopathic medicine for hypertension, bruises and rheumatoid arthritis, and there are also reports of the internal use of the steeped fresh pine needles.[2] However, no known pharmacological indications substantiate these medicinal usages.

In order to develop new drugs derived from natural medicine, over 1500 types of herbs and other plants have been examined. In this study, the anti-serotonergic effect of pine was examined, which has significance in inflammation and in the contraction of blood vessels. It was shown that a hexane-acetone extract of fresh pine leaves inhibits the contractile response induced by serotonin (5-HT) in the rat thoracic aorta. In this report, the active constituent of the extract was examined.

MATERIALS AND METHODS

Fractionation of the Active Anti-serotonergic Constituent in Pines and Pine-Hexane:Acetone (1:1) Extract —— Pine leaves of Japanese red pine and black pine were collected in October. They were dried, coarsely cut and processed using 5 volumes of hexane-acetone (1:1) for cold-extraction. The cold extraction procedure was repeated 3 times. The solvent in the extract was then removed under reduced pressure and the extract was dried by evaporation. The percentage yield of the extract was 2.8%.

In order to examine the active constituent in hexane-acetone (1:1) extract that showed the anti-serotonergic effect, the hexane-acetone extract was further fractionated into 5 fractions by silica gel column chromatography (elution fluid : benzene : acetone = 30 : 1). Fraction 4 was further sepa-

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rated by silica gel column chromatography (elution fluid : benzene : acetone = 10 : 1) and a compound, identified as authentic polyprenol[3] based on mp, IR and NMR data, was used for the experiment.

Male Wistar rats weighing about 250 g were killed by a blow on the head. After bleeding, the chest was opened to remove the thoracic aorta. After the excess fat and connective tissue were removed, the aorta was cut into helical strips about 2 mm wide and 20 mm long.

The composition of modified Kreb's Ringer's solution was as follows (mM) : NaCl 120.4, KCl 4.8, $MgSO_4$ 1.3, $CaCl_2$ 1.2, KH_2SO_4 1.2, $NaHCO_3$ 25.2 and glucose 5.8.

Experimental Animals —— Male Wistar rats (Kitayama Labes) were kept at constant temperature (23 ± 2°C) and humidity (55 ± 5%) for over one week. Healthy animals were selected for the experiments.

Method —— Each aortic strip was placed in a tissue bath containing 25 ml of modified Kreb's Ringer's solution, maintained at 37°C and oxygenated with a gas mixture of 95% O_2 and 5% CO_2. An initial stretch tension of 1 g was applied to each strip and at least one hour was allowed before the start of the experiments. The dose response curve of 5-HT was obtained by addition of 5-HT to the bath cumulatively at concentrations of 10^{-8}, 10^{-7}, 10^{-6}, 5×10^{-6} and 10^{-5} M. Each tissue was then washed 3 times every 10 min. Thirty minutes thereafter, test drugs were applied to the bath. The dose-response curve of 5-HT was again obtained 20 min after the application of test drugs. Ketanserin tartrate served as the control drug. The contractile, response to 5-HT was compared to the maximum response obtained in the first dose response curve of 5-HT taken as 100%. The contracile response was measured with a force-displacement transducer (Nihon Denki Sanei:45196A) and recorded on an oscillograph (Nihon Denki Sanei).

Statistical Analysis —— Statistical analysis was performed with Student's t-test.

RESULTS AND DISCUSSION

In addition to the aforementioned medicinal properties of pine leaves of Chinese White pine,[2] the Chinese Natural Medicine Handbook[4] states that "for stroke, obtain juice of 40 green leaves of pine by pounding, soaking in about half a gallon of wine overnight, soaking it for an additional night placed next to fire, at first drink half a cup of it and later a full cup." It is also written in the Book of Secret Methods[5] that "for injuries, cut up raw pine leaves, grind them, mix them with wine and drink it." These writings suggest that pine leaves have practical medicinal properties, although they are not used in Japan.

In this report, the active ingredients of pine leaves were examined for their inhibitory action on the contractile response to 5-HT in the isolated rat aorta. The tissue did not respond to the extract, but in the examination of the anti-serotonergic action of the hexane:acetone (1:1) extract and each fraction, it was shown that the extract and the fraction which was found to be polyprenol exhibited an anti-serotonergic effect, an effect not known before (Figs. 1, 2). The activity was weaker

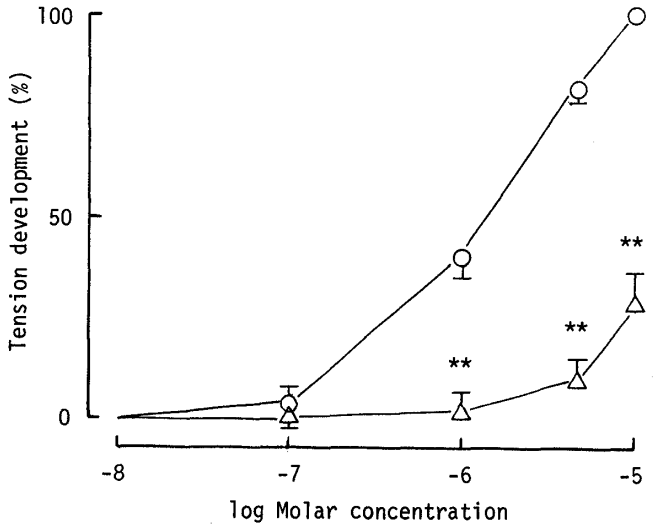

Fig.1. Effect of the Hexane:Acetone(1:1) Ext. of Pine Leaves (Pinus densiflora and P.Thunbergii) on the 5-HT(5-Hydroxytryptamine)-induced Contraction in the Rat Aorta

—○— : control,

—△— : the hexane:acetone(1:1) ext. of pine leaves 5×10^{-2} mg/ml.

Significantly different from control at $*p < 0.05$, $**p < 0.01$.

Each point represents the mean with s.e. of 6 experiments.

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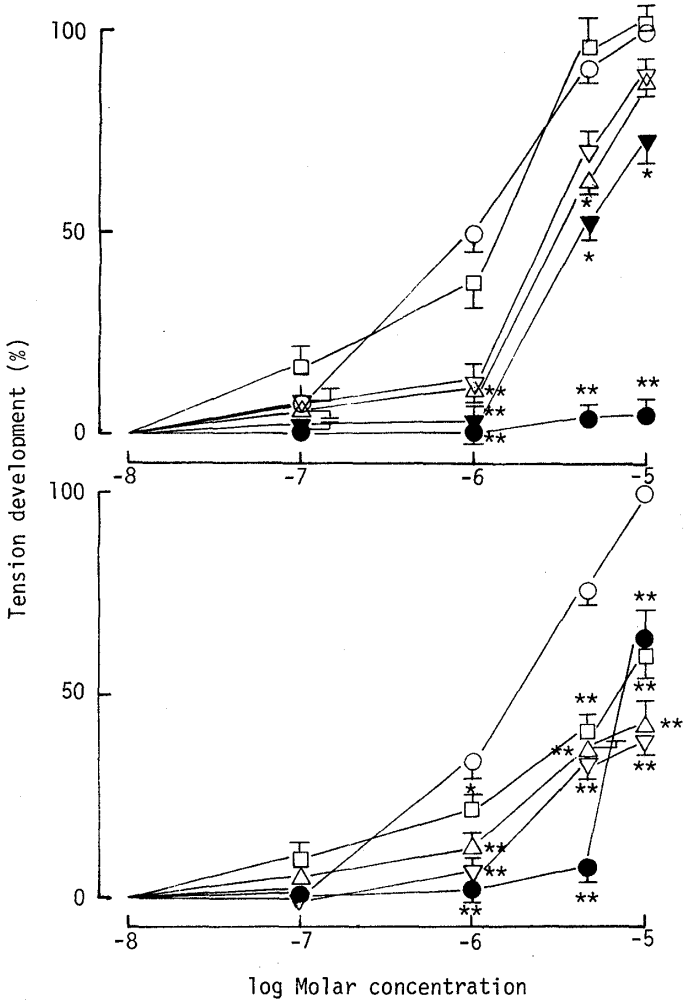

Fig.2. Effects of Fractions of the Hexane: Acetone(1:1) Ext. of Pine Leaves on the 5-HT-induced Contraction in the Rat Aorta
—○— : control, —▽— : fraction I,
—△— : fraction II, —□— : fraction III,
—●— : fraction IV, —▼— : fraction V.
Dose of all fractions is 5×10^{-2} mg/ml.
Significantly different from control at
*$p < 0.05$, **$p < 0.01$.
Each point represents the mean with s.e. of 6 experiments.

Fig.3. Effects of Polyprenol and Ketanserin tartrate on the 5-HT-induced Contraction in the Rat Aorta
—○— : control,
—▽— : polyprenol 10^{-5} M,
—△— : polyprenol 10^{-6} M,
—□— : polyprenol 10^{-7} M,
—●— : ketanserin tartrate 3×10^{-8} M.
Significantly different from control at
*$p < 0.05$, **$p < 0.01$.
Each point represents the mean with s.e. of 6 experiments.

than that of ketanserin (Fig. 3). No effects were found in other autacoids, norepinephrine, histamine, and acetylcholine. This provides a pharmacological basis for one of the traditional medicinal effects of Pinus sp.. Further investigation of the anti-serotonergic action and hemodynamics of polyprenol are currently in progress.

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REFERENCES

1) T.Makino, "Makino's New Illustrated Flora of Japan," ed. by F.Maekawa, Hokuryukan Co.,Ltd., Tokyo, 1962.
2) The Committee of Illustrated Cyclopedia of Medicinal Herbs of China, "Illustrated Cyclopedia of Medicinal Herbs of China," Vol.4, Yuukonsha Co.,Ltd., Kyoto, 1985, pp.685-686.
3) K.Ibata, M.Mizuno, Biochem. J., 213, 305-311 (1983) ; K.Ibata, Mizuno, Phytochemistry, 23, 4, 783-786 (1983).
4) K.Akamatsu, "Wakanyaku," Ishiyaku-shuppan Co.,Ltd., Tokyo, 1970, pp.664-667.
5) B.Isawa, "Illustrated Cyclopedia of Medicinal Plants of Japan," Seibundo-shinkosha Publishing Co.,Ltd., Tokyo, 1980, pp.219-220.

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