Journal of Traditional and Complementary Medicine 6 (2016) 140–145

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original article

Sedative effects of inhaled essential oil components of traditional fragrance *Pogostemon cablin* leaves and their structure–activity relationships

Ken Ito [a, b, c, *], Yasuko Akahoshi [a], Michiho Ito [a], Shuji Kaneko [d]

[a] *Department of Pharmacognosy, Graduate School of Pharmaceutical Science, Kyoto University, Sakyo-ku, Kyoto, Japan*
[b] *Division of Medicinal Chemical Sciences, Pharmacognosy, Kyoto Pharmaceutical University, Yamashinaku, Kyoto, Japan*
[c] *The Museum of Osaka University, Osaka University, Toyonaka, Japan*
[d] *Department of Molecular Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan*

ARTICLE INFO

Article history:
Received 5 December 2014
Received in revised form
8 January 2015
Accepted 9 January 2015
Available online 23 February 2015

Keywords:
behavioral pharmacology
diacetone alcohol
inhalation
Labiatae
Pogostemon cablin
sedative effect
structure–activity relationship

ABSTRACT

Plants rich in essential oils, such as *Pogostemon cablin* (*P. cablin*; 廣藿香 guǎng huò xiāng), have been used for aromas and as herbal medicines since ancient times because of their sedative effects. We investigated the sedative effects of hexane extract from *P. cablin* using locomotor activity in mice. Inhalation of *P. cablin* hexane extract exhibited significant sedative activity in a dose-dependent manner. In order to isolate the active constituents, the extract was fractionated and diacetone alcohol was identified as an active compound. Inhalation of diacetone alcohol significantly reduced murine locomotor activity in a dose-dependent manner, and this effect was not observed in olfaction-impaired mice. We examined the structure–activity relationship of diacetone alcohol and similar compounds. The ketone group at the two-position and number of carbons may play important roles in the sedative activity of diacetone alcohol.

1. Introduction

In Japan, there is a traditional custom of burning incense or enjoying scents from scent bags, which are made of Japanese paper or cloth containing aromatic herbal medicines and cause the scent to be emitted for a long time without the use of a flame. Typically, mixtures of minced herbal medicines have been used, such as dried herbs of *Pogostemon cablin* (*P. cablin*; 廣藿香 guǎng huò xiāng), bark of *Cinnamomum cassia* (肉桂 ròu guì), heartwood of *Santalum album* (檀香 tán xiāng), and roots of *Hedychium spicatum* (草果藥 cǎo guǒ yào), *Illicium verum* (八角 bā jiǎo), *Syzygium aromaticum* (丁香 dīng xiāng), *Nardostachys chinensis* (甘松 gān sōng), and *Dryobalanops aromatica* (冰片 bīng piàn). The extracts of these herbal medicines have been reported to have insecticidal and bactericidal potentials.[1,2] However, the effects of the scents, that is, inhaled volatile components, have not been sufficiently examined. Recently, aromatherapy, in which unhealthy people inhale essential oil components may prevent or treat physical and mental ailments, has received considerable attention. We conducted simple experiments using mice to examine the aromatic components of *P. cablin* and their sedative effects. *P. cablin* is also blended in the formula of traditional Chinese medicine (中醫 zhōng yī; TCM) and frequently used for its aroma. In previous studies, the scavenging effects of reactive oxygen species,[3] antitrypanosomal activities[4] of the extract, and decreases of human relative sympathetic activities due to inhaled oil have been reported.[5] In this study, we evaluated the effects of inhalation of the extract of *P. cablin* and its active components on mouse locomotor activity. Additionally, we examined the structure–activity relationships of the active component, diacetone alcohol.

2. Materials and methods

2.1. Materials

Dried leaves of *P. cablin* (廣藿香 guǎng huò xiāng) were purchased from Mitsuboshi Pharmaceutical Co. Ltd. (Nara, Japan).

* Corresponding author. Misasagi-Shichonocho 1, Yamashinaku, Kyoto 607-8412, Japan.
E-mail address: itoken@mb.kyoto-phu.ac.jp (K. Ito).

Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2015.01.004

Lavender oil used as a positive control for the inhalation studies was purchased from Nacalai Tesque, Inc. (Kyoto, Japan) and its batch number was the same as that in our previously reported studies.[6–8] Gas chromatography–mass spectrometry (GC-MS) analysis showed that this lavender oil contained 41.3% linalool and 48.2% linalyl acetate.[8] Triethyl citrate (Merck, Darmstadt, Germany), an odorless solvent, was used to dissolve the scent components. Chlorpromazine hydrochloride and pentobarbital were obtained from Mitsubishi Tanabe Pharma Corporation (Osaka, Japan). Diacetone alcohol, 1,1-dimethyl-1-butanol, isobutyl methyl ketone, 4-hydroxy-2-butanone, zinc sulfate, and corn oil were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Animals

Animal experiments were designed following recommendations from the Animal Research Committee of Kyoto University, Kyoto, Japan. Experimental procedures involving the animals and their care were conducted in conformity with the institutional guidelines that are in compliance with Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science, and Technology, Japan (2006). Male 4-week-old ddY mice (25–30 g) were purchased from Japan SLC (Shizuoka, Japan). The mice were housed in colony glass cages at a temperature of 25 °C and a relative humidity of 50–60% with a light–dark cycle of 12 hours prior to being used for the experiments. They were fed standard pellet chow and water *ad libitum*. All behavioral observations were conducted between 10:00 and 17:00. The sedative activity of the scent components was evaluated in mice assessing their spontaneous motor activity in an open-field test as described in our previous report.[6] Briefly, fragrance components were dissolved in triethyl citrate (400 μL total), and four filter paper disks were permeated with the solution. The disks were placed on the wall of a glass cage (W 60 cm × L 30 cm × H 34 cm) using Scotch tape, and the vapor of the solution pervaded the glass cage by natural diffusion. Sixty minutes after charging the solution, a mouse was placed into the center of the glass cage and was monitored by a video camera for another 60 minutes. In a further experiment, diacetone alcohol was dissolved in corn oil and intraperitoneally injected into mice. The frequency of the mouse crossing lines drawn on the bottom of the glass cage at 10 cm intervals was determined every 5 minutes for 60 minutes. The area under the curve representing total locomotor activity for 60 minutes was calculated by the trapezoidal rule. Another experiment using pentobarbital was conducted in accordance with our previous paper.[8]

2.3. Olfaction impairment of mice by zinc sulfate treatment

Zinc sulfate treatment was conducted as previously reported.[9] Nasal drops (0.1 mL) of 1% zinc sulfate solution were carefully administered through a guide cannula only to the left nasal cavity of 4-week-old male ddY mice in a dorsal position under pentobarbital anesthesia. To achieve sufficient effects on the olfactory epithelium, mice received the nasal drops of zinc sulfate solution and were kept in a dorsal position for 10 minutes. Two days after the treatment, mice with sufficiently impaired olfaction were used in the experiment. The impaired olfaction was confirmed by an olfaction test using acetic acid. The test was conducted as previously reported.[10] Cotton wool with 500 μL of 100% acetic acid dropped onto it was placed at one end of a glass cage. Cotton wool with 500 μL of distilled water dropped onto it was placed at the other end of the glass cage. The glass cage bottom was separated into halves by drawing a line. The behaviors of the mice in this glass cage were observed for 15 minutes. Then, the time spent in the acetic acid or distilled water area was recorded.

2.4. GC-MS analysis

Qualitative analysis of volatile components was conducted as follows: GC-MS analysis of the compounds in vapor was performed on a G7000-M9000/3DQMS (Hitachi, Tokyo, Japan) under the following operating conditions: column: fused silica capillary column TC-WAX (Hewlett Packard, Palo Alto, CA, USA), 60 m × 0.25 mm, 0.25 μm film thickness; column temperature: 40–120 °C increasing at 16 °C/min, 5 minutes at 120 °C, 120–130 °C increasing at 1 °C/min, 15 minutes at 130 °C, 130–200 °C increasing at 20 °C/min, 20 minutes at 200 °C; carrier gas: He, 147.1 kPa; and ionization energy: 15 eV. The methods used for the adsorption and desorption of the volatile components using solid-phase microextraction were described in a previous report.[11] GC-MS analysis of the compounds present in the examined liquid was performed on a Hewlett Packard 5890 (Hewlett Packard) and AUTOMASS (JEOL, Tokyo, Japan) under the following operating conditions: column: fused silica capillary column TC-WAX (Hewlett Packard), 60 m × 0.25 mm, 0.25-μm film thickness; column temperature: 40–130 °C increasing at 2 °C/min, 25 minutes at 130 °C, 130–140 °C increasing at 2 °C/min, 15 minutes at 140 °C, 140–200 °C increasing at 15 °C/min, 30 minutes at 200 °C; injector: 180 °C, carrier gas: helium, 45 cm/min; column head pressure: 100 kPa; injection volume: 1 μL; and ionization energy: 70 eV. The chemical components were identified by comparison with the retention times, mass and ion spectra from an MS data library (NIST 02), of authentic standard compounds.

2.5. Lavender oil

The lavender oil used in this study was from Nacalai Tesque, Inc (Kyoto, Japan), and was analyzed by GC and GC-MS to identify the main components. GC-MS analysis was performed on a Hewlett Packard 5890 (Hewlett Packard) connected with AUTOMASS (JEOL) with the above-mentioned operating conditions. Chemical components were identified by comparing their retention time and mass spectra with those in the MS data library (NIST 02), and authentic standards.

2.6. Measurement of evaporated compound in a glass cage

A compound dissolved in triethyl citrate was dropped onto filter paper. Then, the filter paper was weighed and placed in a closed glass cage. The filter paper was removed at 1 hour and weighed again. Weight difference from that immediately after dropping was regarded as the amount of compound that evaporated per hour in the glass cage.

2.7. Extraction of P. cablin leaves and fractionation of their oil

Dried leaves of *P. cablin* (5.0 kg) were extracted with 2 L of hexane by simply standing for 2 days and the solvent was evaporated in reduced pressure to yield 198 g of essential oil. The oil (13.7 g) was fractionated by silica gel column chromatography (7 cm inner diameter × 25 cm) eluting with a solvent mixture of hexane-AcOEt (4:1) to yield Fraction 1 (4.3 g), Fraction 2 (5.3 g), and Fraction 3 (3.4 g).

2.8. Evaluation of lipophilicity

The lipophilicity of compounds was examined using Marvin Sketch (ChemAxon, Budapest, Hungary) on the basis of Log P values, a common logarithm of n-octanol/water partition coefficient.[12]