Pungent Qualities of Sanshool-Related Compounds Evaluated by a Sensory Test and Activation of Rat TRPV1

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The detection threshold and taste characteristics of sanshools were examined by sensory evaluation, after isolating four sanshools (α-, β-, γ-, and δ-), and two hydroxy sanshools (α- and β-) from the pericarp of Japanese pepper. The Scoville unit (SU) values of the four sanshools were in the range of 80,000–110,000, while those of hydroxy sanshools were 3–5 fold lower than corresponding sanshools. The pungent qualities of each sanshool were different. Burning and tingling were predominantly perceived and lasted for the longest time with α-sanshool. Burning and fresh for γ-sanshool, and tingling and numbing for hydroxy α-sanshool were perceived. Tests on the activation of rat TRPV1 were also performed. All of them were weak agonists. Among them, γ-sanshool was the most potent agonist, although its EC50 value of 5.3 µM was 230 fold higher than that of capsaicin. These results indicate that it would be difficult to explain the pungent quality of each sanshool simply in terms of TRPV1 activation.

Key words: Japanese pepper; sanshool; Scoville unit; pungency; rat TRPV1

Japanese pepper (Xanthoxylum piperitum DC.) is widely utilized in Japan as a spice for its pleasant flavor and delectable pungency. Crombie has confirmed the structures of α- and β-sanshools1,2 as the pungent principles in Japanese pepper. Many studies3–6 have subsequently been conducted to isolate and identify other new types of sanshool such as γ-sanshool, and hydroxy α-, β-, and γ-sanshools from the pericarp of Japanese pepper and from huajiao, the pericarp of Zanthoxylum bungeanum Maxim (Rutaceae), which grows in Sichuan, China. More recently, Kashiwada et al.7 have isolated and determined the structures of six sanshools, including newly identified (2E,4E,8E,10E,12E)-N-isobutyl-2,4,8,10,12-tetradecapentaenamide (the all-trans isomer of γ-sanshool) from the fruit of Japanese pepper. They have reported that hydroxy α- and hydroxy γ-sanshools, having one cis and three or four trans double bonds in the molecule, are strongly pungent, while hydroxy β-sanshool, having all-trans double bonds, is tasteless.6)

A number of reports have been published on the taste and detection threshold of the pungent principles from such other plant species as hot pepper, pepper, ginger, galangal, and myoga5,7–10 but little is known about the characteristics of the taste of sanshools, because authentic samples are available only by isolating natural sanshool from plants or by synthesis of it.

Recent reports indicate that certain pungent principles such as capsaicin, gingerol, and piperine activate the transient receptor potential vanilloid type 1 (TRPV1).11,12 This receptor is a non-selective cation channel,11 and is distributed in the trigeminal neurons of the oral cavity.13 The pungent sensation is evoked through activation of the sensory neurons when this ion channel is opened.13 But, it is unclear whether sanshools would induce the opening of this ion channel.

We isolated pure sanshools in the present study as authentic samples from dried pericarps by silica gel chromatography and semi-preparative HPLC. These samples were examined by sensory evaluation to identify the detection threshold and taste of each sanshool. We also examined whether the pure sanshools would directly open the TRPV1 ion channel heterologously expressed in HEK293 cells.

Materials and Methods

Materials. Authentic sanshools were obtained from the dried pericarp of ripe fruits of Japanese pepper purchased from Arida JA (the Japanese Agricultural Cooperative Organization) in Wakayama Prefecture in 2003.

Isolation of authentic sanshools. α-, β- and γ-sanshools and (2E,4E,8E,10E,12E)-N-isobutyl-2,4,8,10,12-tetradecapentaenamide, the all-trans isomer of γ-sanshool, and hydroxy α- and β-sanshools were isolated as authentic samples. The all-trans isomer of γ-sanshool is referred to as δ-sanshool in this paper.

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(Fig. 1). Ground dried pericarp (50 g) was soaked overnight in diethyl ether (1000 ml). This procedure was conducted twice. After filtration, the combined filtrate was concentrated *in vacuo* under nitrogen gas. The extract obtained was fractionated by silica gel chromatography, using a stepwise change in the ratio of hexane–EtOAc as the eluent. Four sanshools, and hydroxy α- and β-sanshools, were eluted respectively in the 50% and 50%–100% EtOAc/hexane fractions. To isolate each sanshool compound separately, each sanshool fraction was applied to semi-preparative HPLC in an ODS column (Senshu Pak, Pegasil ODS, 250 mm; Senshu Scientific, Tokyo, Japan) with a PR201 recycle bulb set as the eluent. Four sanshools, and hydroxy α- and β-sanshools, were eluted respectively in the 5% sucrose solution containing both EtOH and propylene glycol was designated the blank, and the panelists for the sensory evaluation could perceive both the taste and smell of EtOH at a 1% concentration, and its smell at 0.1%. The final concentration of propylene glycol used for dissolving each sanshool in the 5% sucrose solution was 0.6%. A sanshool solution was prepared immediately before the evaluation and kept at room temperature. The propylene glycol used was of food additive grade, and EtOH was of Japanese Pharmacopoeia grade. Granulated sugar was used, because it has the highest sucrose purity of all the kinds of edible sugar on the market. We also used commercial mineral water manufactured by the same company (Natural Mineral Water; Suntory, Osaka, Japan) throughout this study.

**Table discussion.** Fourteen female panelists aged 22–26 participated in this study. Prior to their evaluation, all the panelists were confirmed to have normal taste sensitivity for the detection threshold of capsaicin which is 8.6 ± 2.7 × 10^6 g/ml, *i.e.* 1.20 × 10^7 Scoville units (SU, representing the reciprocal of the concentration of a sample solution).

The panelists took part in a table discussion session to determine the evaluation terms. They were requested to describe perceived sensations according to the list of terms by Koido, and the terms finally used were selected after consensus was reached among all the members.

**Determination of the detection threshold.** Pungency was evaluated using modified ASTA methodology with a paired difference test. The concentration of a solution at which everyone could perceive pungency was diluted in a binary manner with a 5% sucrose solution. The 5% sucrose solution containing both EtOH and propylene glycol was designated the blank, and the discernible minimum concentration was designated the detection threshold. The sanshool solution and rinsing water were kept at room temperature, because it has been reported that the burning sensation induced by capsaicin is enhanced by warming and inhibited by cooling. The panelists swirled the entire sample

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**Fig. 1.** Structures of the Sanshool Compounds Investigated in This Study.


structure of each compound was confirmed by comparing the NMR and EI-MS data with those reported by Yasuda et al. and Mizutani et al.

The purity of each compound analyzed by HPLC was as follows: α-sanshool, 99.8%; β-sanshool, 95.7%; γ-sanshool, 99.7%; δ-sanshool, 97.8%; hydroxy α-sanshool, 99.8%; and hydroxy β-sanshool, 99.3%.

**Sensory evaluation.**

**Sample preparation.** The sensory test and determination of the detection threshold of pungency were conducted on an EtOH solution of each sanshool dissolved in propylene glycol prior to its dilution with a 5% sucrose solution, because sanshools are insoluble in water but soluble in EtOH and propylene glycol. A high concentration of EtOH gives some stimulus to the tongue, so propylene glycol was mixed with EtOH. The final concentration of EtOH was set at 0.1% (v/v), because the panelists for the sensory evaluation could perceive both the taste and smell of EtOH at a 1% concentration, and its smell at 0.1%. The final concentration of propylene glycol used for dissolving each sanshool in the 5% sucrose solution was 0.6%. A sanshool solution was prepared immediately before the evaluation and kept at room temperature. The propylene glycol used was of food additive grade, and EtOH was of Japanese Pharmacopoeia grade. Granulated sugar was used, because it has the highest sucrose purity of all the kinds of edible sugar on the market. We also used commercial mineral water manufactured by the same company (Natural Mineral Water; Suntory, Osaka, Japan) throughout this study.
(5 ml) in their mouths for 10 s, expectorated, waited for 60 s, and then evaluated which was more pungent while explaining the taste of the sample they had just evaluated. They were requested to evaluate two samples at each session, to rinse their mouths with mineral water, and to wait for 5 min between samples. The ASTA 21.0 method(15) for capsaicin evaluation requires each panelist to test a series of capsaicin solutions from low to high concentration until pungency can be perceived. In this study, the stimulus from sanshool lasted for several minutes, so that only two samples were examined during one session.

Characterization of pungency. To further characterize the taste of each sanshool, the pungency of an EtOH solution of the sanshool sample (0.25 mg/ml) was evaluated. Each panelist soaked a cotton bud with about 0.1 ml of the sample solution, and then rubbed it on the tip and both sides of the tongue. The panelist measured the duration of the stimulus up to 10 min. Duration was analyzed by Student’s t-test for interaction between any two samples. The intensity of α- and γ-sanshool samples was also determined by means of a comparative duo test. The sample solution was composed of 5% sucrose, 0.4% EtOH containing the sanshool sample, and 2.5% propylene glycol. The final concentration of α- or γ-sanshool was 2.0 x 10^{-4} g/ml. At the start of the session, each panelist rinsed the mouth with water and expectorated. The comparative duo test involved an aliquot (0.1 ml) of the sample solution containing α-sanshool applied with a syringe on one side of the tongue, whereas the sample solution containing γ-sanshool was applied on the other side of the tongue. The panelist was asked to leave the sample solutions for 10 s and then expectorate, before waiting for 60 s to identify only the “burning” sensation by a comparison of both sides and giving a score for the burning stimulus on each side of the tongue. The scores were defined as follows: zero, not detectable; 1, barely detectable; 2, detectable; 3, strongly detectable. Intensity was analyzed by Student’s t-test for interaction between two samples. All statistical analyses were conducted using SPSS 12.0 J. (SPSS, 2003, Tokyo, Japan)

Measurement of the intracellular Ca^{2+} concentration in TRPV1-expressing HEK293 cells. We measured the intracellular Ca^{2+} concentration essentially according to the methods reported by Catelina et al. (11) Rat TRPV1 cDNA was amplified by RT-PCR, using mRNA obtained from rat C6 glioma cells. This cDNA was cloned into pcDNA3 (Invitrogen, CA, U.S.A.), and then transfected into HEK293 cells by using the SuperFect transfection reagent (Qiagen, Hilden, Germany). After culturing in the presence of G418, we obtained HEK293VR11 cells, which stably express rat TRPV1. HEK293VR11 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 250 ng/ml of amphotericin B at 37°C under 5% CO_{2}/air. The cells were sub-cultured every week; the highest passage number used was 35.

HEK293VR11 cells were loaded with the cytoplasmic calcium indicator, Fluo-4-AM (2.5 μM; Molecular Probes, OR, U.S.A.) in a loading buffer (5.37 mM KCl, 0.441 mM KH_{2}PO_{4}, 0.406 mM MgSO_{4}–7H_{2}O, 137 mM NaCl, 0.336 mM Na_{2}HPO_{4}–7H_{2}O, 5.56 mM Glucose, 20 mM HEPES, 0.492 mM MgCl_{2}–6H_{2}O, 2.26 mM CaCl_{2}, and 0.1% bovine serum albumin at pH 7.4). The time-dependent change in fluorescence (excitation wavelength of 485 nm, emission wavelength of 540 nm) was recorded with a CAF-110 fluorospectrophotometer (Jasco, Tokyo, Japan), and analyzed using PowerLab (AD Instruments, NSW, Australia). Each data value was expressed as a percentage of the response to 10 μM capsaicin. Curve fitting and parameter estimation were carried out using Prism4 (Graph Pad Software, CA, U.S.A.).

Results

Isolated sanshool compounds

The structures of the four isolated sanshools (α to δ) and the two hydroxy sanshools (α and β) were confirmed by spectral analyses, and are shown in Fig. 1. α-Sanshool is referred to as (2E,6Z,8E,10E)-N-isobutyl-2,6,8,10-dodecataeraneamide, which includes three trans and one cis double bonds, in the molecule. β-sanshool is the all-trans isomer of this compound. Hydroxy α- and hydroxy β-sanshools respectively have a hydroxy group at the 2'-position of α-sanshool and β-sanshool. γ-Sanshool is referred to as (2E,4E,8Z,10E,12E)-N-isobutyl-2,4,8,10,12-tetradecapentaenamide, with a side chain whose carbon number is 14 and with four trans and one cis double bond in the molecule.

Sensory evaluation

Detection threshold values for the sanshools and hydroxy sanshools

It was found from a preliminary experiment that the stimulus by the solution containing each sanshool remained 30 s after a panelist had expectorated the sample solution. The panelists were therefore instructed to evaluate a sample 60 s after they expectorated, and to leave an interval of 5 min between samples.

The distributions of the detection threshold and the Scoville units (SU) of the stimuli from the four sanshools, two hydroxy sanshools, and capsaicin examined by sensory evaluation are listed in Table 1. The filled circle in a line shows one panelist’s threshold. A 4-step dilution series of the sample capsaicin solution from 18.0 x 10^{-8} g/ml (step 1) to 0.3 x 10^{-8} g/ml (step 4) was prepared and presented to each panelist starting with step 1. This dilution series was monitored by the method described by Sizer et al. (17) In respect of capsaicin, the largest number of panelists evaluated the threshold value as 9.0 x 10^{-8} g/ml. Although the reported detection threshold for capsaicin is 6.3 x 10^{-8} g/ml (1.6 x 10^{7} SU), (8) the result that the largest
Table 1. Detection Threshold Values for the Sanshool Compounds and Capsaicin

<table>
<thead>
<tr>
<th>Concentration (×10⁻⁸ g/ml)</th>
<th>Sanshool</th>
<th>Hydroxy sanshool</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α</td>
<td>β</td>
</tr>
<tr>
<td>20.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.625</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.312</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean threshold value</td>
<td>1.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Scoville (×10⁵ SU, ml/g) 0.80 0.70 1.10 1.10

Table 2. Pungent Qualities of the Sanshool Samples in a 5% Sucrose Solution

<table>
<thead>
<tr>
<th>Pungent compounds</th>
<th>Pungent qualities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanshool</td>
<td>α burning, tingling, numbing</td>
</tr>
<tr>
<td></td>
<td>β numbing, bitter</td>
</tr>
<tr>
<td></td>
<td>γ burning, numbing, fresh, bitter</td>
</tr>
<tr>
<td></td>
<td>δ burning, numbing, fresh</td>
</tr>
<tr>
<td>Hydroxy sanshool</td>
<td>α tingling, numbing</td>
</tr>
<tr>
<td></td>
<td>β numbing, astringent, bitter</td>
</tr>
</tbody>
</table>

Characterization of pungency

The panelists were instructed to describe the taste of the samples evaluated during the sensory test to determine the threshold values; the results are summarized in Table 2. The pungent qualities of the sanshool samples in the 5% sucrose solution were as follows: α-sanshool, burning, tingling, and numbing; β-sanshool, numbing and bitter; γ-sanshool, burning, numbing, fresh, and bitter; and δ-sanshool, burning, numbing, and fresh. The pungent qualities of the hydroxy sanshool samples were a little different from the sanshool samples: hydroxy α-sanshool, tingling and numbing; and hydroxy β-sanshool, numbing, astringent, and bitter. “Burning” (piri piri in Japanese) was defined in the table discussion as the perception of being pricked by a thick needle, and “tingling” (hiri hiri in Japanese) as the lasting perception of being pricked by a fine needle. “Fresh” includes both the fresh sensation after swirling the sample solution and the cool sensation caused by numbing of the tongue.

To characterize the pungency of the sanshool compounds, the duration of the stimulus was also measured; the results are shown in Fig. 2. The panelists were instructed to state how long the stimulus lasted from the EtOH solution of each compound (0.25 mg/ml). The effect of α-sanshool lasted significantly longer than the other three sanshools and two hydroxy sanshools. As described in “Materials and Methods”, although EtOH slightly stimulated the tongue, the panelists perceived the pungent characteristics of the sanshool samples.

The intensity of the burning sensation by α- and γ-sanshool samples was measured in the dilution series of each sanshool sample solution from 20 × 10⁻⁵ g/ml (step 1) to 0.156 × 10⁻⁵ g/ml (step 8) and presented to the panelists starting from step 1 for hydroxy α- and β-sanshool, from step 2 for α- and γ-sanshool, and from step 3 for β- and δ-sanshool. The respective detection thresholds for α- and β-sanshools were in the range of 0.312–2.5 × 10⁻⁵ g/ml. The largest number of panelists evaluated the threshold values for γ- and δ-sanshools as 0.312 × 10⁻⁵ g/ml. As for hydroxy α-sanshool and β-sanshool, the respective threshold values were in the range of 1.25–10.0 × 10⁻⁵ and 5.0–20.0 × 10⁻⁵ g/ml, higher than the former four types of sanshool. Each average threshold value was determined as the detection threshold for the stimulus, and its SU value was calculated using the average value. The average threshold values for the four sanshools and two hydroxy sanshools were 1.3, 1.4, 0.9, 0.9, 3.8, and 7.8 × 10⁻⁵ g/ml, respectively. Although the four sanshools had similar SU values, the hydroxy sanshool tended to be higher than the other three sanshools and two hydroxy sanshools.

Characterization of pungency

The panelists were instructed to describe the taste of the samples evaluated during the sensory test to determine the threshold values; the results are summarized in Table 2. The pungent qualities of the sanshool samples in the 5% sucrose solution were as follows: α-sanshool, burning, tingling, and numbing; β-sanshool, numbing and bitter; γ-sanshool, burning, numbing, fresh, and bitter; and δ-sanshool, burning, numbing, and fresh. The pungent qualities of the hydroxy sanshool samples were a little different from the sanshool samples: hydroxy α-sanshool, tingling and numbing; and hydroxy β-sanshool, numbing, astringent, and bitter. “Burning” (piri piri in Japanese) was defined in the table discussion as the perception of being pricked by a thick needle, and “tingling” (hiri hiri in Japanese) as the lasting perception of being pricked by a fine needle. “Fresh” includes both the fresh sensation after swirling the sample solution and the cool sensation caused by numbing of the tongue.

To characterize the pungency of the sanshool compounds, the duration of the stimulus was also measured; the results are shown in Fig. 2. The panelists were instructed to state how long the stimulus lasted from the EtOH solution of each compound (0.25 mg/ml). The effect of α-sanshool lasted significantly longer than the other three sanshools and two hydroxy sanshools. As described in “Materials and Methods”, although EtOH slightly stimulated the tongue, the panelists perceived the pungent characteristics of the sanshool samples.

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Characterization of pungency

The panelists were instructed to describe the taste of the samples evaluated during the sensory test to determine the threshold values; the results are summarized in Table 2. The pungent qualities of the sanshool samples in the 5% sucrose solution were as follows: α-sanshool, burning, tingling, and numbing; β-sanshool, numbing and bitter; γ-sanshool, burning, numbing, fresh, and bitter; and δ-sanshool, burning, numbing, and fresh. The pungent qualities of the hydroxy sanshool samples were a little different from the sanshool samples: hydroxy α-sanshool, tingling and numbing; and hydroxy β-sanshool, numbing, astringent, and bitter. “Burning” (piri piri in Japanese) was defined in the table discussion as the perception of being pricked by a thick needle, and “tingling” (hiri hiri in Japanese) as the lasting perception of being pricked by a fine needle. “Fresh” includes both the fresh sensation after swirling the sample solution and the cool sensation caused by numbing of the tongue.

To characterize the pungency of the sanshool compounds, the duration of the stimulus was also measured; the results are shown in Fig. 2. The panelists were instructed to state how long the stimulus lasted from the EtOH solution of each compound (0.25 mg/ml). The effect of α-sanshool lasted significantly longer than the other three sanshools and two hydroxy sanshools. As described in “Materials and Methods”, although EtOH slightly stimulated the tongue, the panelists perceived the pungent characteristics of the sanshool samples.

The intensity of the burning sensation by α- and γ-
sanshool at a concentration of $2 \times 10^{-4}$ g/ml in a 5% sucrose solution was also examined because their detection threshold values were similar, while the duration of the stimulus by $\alpha$-sanshool was significantly longer than that by $\gamma$-sanshool. The results are shown in Table 3. The average score for the intensity of burning induced by $\alpha$-sanshool was 2.8 ± 0.4. This was significantly stronger than that by $\gamma$-sanshool (0.6 ± 0.8). These results show that the panelists perceived the intensity of burning induced by $\alpha$-sanshool as being much stronger than that by $\gamma$-sanshool.

**Activation of TRPV1**

TRPV1 is a cation channel distributed in a subpopulation of warm-sensitive neurons which is excited by capsaicin. We examined whether the sanshool samples would activate TRPV1 expressed in HEK293 cells. Figure 3 shows the dose-response curve for capsaicin, the four sanshool samples, and the two hydroxy sanshool samples. Capsaicin increased the intracellular Ca$^{2+}$ concentration dose-dependently in HEK293VR11 cells. Its EC$_{50}$ value was 0.023 μM, almost the same as the values reported by others. All the sanshool and hydroxy sanshool samples increased the intracellular Ca$^{2+}$ concentration, but the potency of each was much lower than that of capsaicin. Therefore, all the sanshool and hydroxy sanshool samples were very weak agonists for TRPV1. Among them, $\gamma$-sanshool was the most potent agonist, although its EC$_{50}$ value of 5.3 μM was 230 fold higher than that of capsaicin. The $\alpha$-, $\beta$-, and $\delta$-sanshool samples and the hydroxy $\beta$-sanshool sample increased the intracellular Ca$^{2+}$ concentration with the

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**Table 3. Intensity of the Burning Stimulus by the $\alpha$- and $\gamma$-Sanshool Samples**

<table>
<thead>
<tr>
<th>Pungent compounds</th>
<th>Burning intensity score</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-sanshool</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>$\gamma$-sanshool</td>
<td>0.6 ± 0.8</td>
</tr>
</tbody>
</table>

Each value is expressed by the mean ± SD (n = 10). Values are significantly different at $p < 0.001$ by Student’s t-test.

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**Fig. 2. Duration of the Stimulus with Each Sanshool Compound.**

The panelists were instructed to measure the duration of the stimulus caused by each sanshool compound up to 10 min (600 s). Each block shows the mean value topped by the SD (n = 12). Values not sharing common superscript letters (a, b, and c) are significantly different at $p < 0.05$ (a and b) and $p < 0.01$ (c) by Student’s t-test.
same potency. But, only the EC₅₀ values for α- and β-
sanshool were calculated respectively as 29 μM and
54 μM, the values for the δ-sanshool and hydroxy α- and
β-sanshool samples were not calculated because of their
low solubility.

Discussion

In the present study we isolated and purified the α-, β-, γ-, and δ-sanshool compounds, as well as the
hydroxy α- and β-sanshool compounds from the dried
pericarp of Japanese pepper, and investigated their
detection threshold values and pungent qualities. As
shown in Tables 2 and 3, the stimulus perceived as
burning was detected mainly in the α- and γ-sanshool
samples among those tested, and the stimulus from α-
sanshool was significantly stronger than that of γ-
sanshool, but, the panelists’ perceived threshold value
for γ-sanshool was lower than that for α-sanshool, as
shown in Table 1. Many panelists sensed a fresh and
bitter taste in the γ-sanshool solution. The stimulus of
hydroxy α-sanshool causing burning and tingling was
stronger and more recognizable than that of hydroxy β-
sanshool. Although Bryant and Mezine[13] have reported
hydroxy β-sanshool in 70% EtOH to be tasteless and
inactive, the panelists participating in this present study
evaluated the effect as numbness with the 5% sucrose
solution of hydroxy β-sanshool.

The four sanshool samples had similar SU values,
while the two hydroxy sanshool samples had lower SU
values than these. In other words, a possible explanation
for the low stimulus provided by these latter two
sanshools is their impermeability across the tongue
epithelium. The higher log P and lower threshold values
for the α- and β-sanshools than those of the hydroxy α-
and hydroxy β-sanshools might indicate greater perme-
ability across the tongue epithelium. The four sanshool
samples (α to δ) had SU values similar to piperine from
pepper (1.0 × 10⁵ SU) and 6-gingerol from ginger
(8.0 × 10⁴ SU). The SU value for capsaicin (1.6 × 10⁵ SU)⁸
was 200 times higher than the values for
these sanshools. Cliff et al.[19] have reported that the
effect of piperine, capsaicin, and ginger oleoresin, whose
concentrations were about 20 fold higher than their
threshold values, except for ginger oleoresin, was
predominantly one of burning, with slight tingling and
numbing sensations. The pungent qualities of these three
compounds might be similar to those of α-sanshool.

The pungent qualities of the four sanshool samples (α
to δ) were also different from each other. While the
pungency of α-sanshool and hydroxy α-sanshool was
perceived mainly as burning and tingling, the pungency
of their two all-trans isomers was numbng and
astringent. Bitterness was perceived with β-, γ-, and δ-
sanshool and with hydroxy β-sanshool, and freshness
with burning was also perceived with the γ- and δ-
sanshool samples. These results suggest that the number
of carbons on the side chain and geometricisomerism
affect the pungency of sanshools, although further study
is required to confirm this. It is thought that the
difference in the threshold values and the pungent
qualities might have been due to the difference in log P
values, which show hydrophobicity. The log P values for
the sanshool samples were then calculated using CS
Chem Draw Ultra Version 6.0 (Cambridge Soft, MA,
U.S.A., 2000), giving the following results: α- and β-
sanshools, 3.93; γ- and δ-sanshools, 4.45; hydroxy α-
and hydroxy β-sanshools, 2.73.

The duration of the stimulus by each sanshool sample
in an EtOH solution is shown in Fig. 2. A significant
difference in stimulus duration was found between α-
and β-sanshool and also between γ- and δ-sanshool.
α-Versus β- and γ-versus δ-sanshools are geometrical
isomers of each other. Although the detection threshold
values for these four compounds were similar, the
duration of the stimulus with each was very different.
These results cannot be explained solely by the log P
values, because the geometrical isomers had the same
calculated log P values.

We then measured the activation of rat TRPV1 by the
four sanshool and two hydroxy sanshool samples. The
pungency of capsaicin was primarily one of burning,
which probably resulted from the activation of TRPV1
in warm-sensitive neurons.[13] All the sanshool samples
activated TRPV1 expressed in HEK293 cells, as shown
in Fig. 3, indicating that the burning sensation arouse
from the activation of TRPV1. But, their potency was
very weak; the EC₅₀ value of 5.3 μM for γ-sanshool,
which was the most potent agonist, was about 200 times
higher than that of capsaicin. As shown in Table 1, the
detection threshold value for γ-sanshool was almost 200
times higher than that for capsaicin. These results lead
us to believe that the burning sensation from γ-sanshool
is to some extent related to the activation of TRPV1.
On the other hand, the EC₅₀ values of 29 μM for α-sanshool
and 54 μM for β-sanshool were five and ten times higher
respectively than that of γ-sanshool, although their
threshold values were only a little higher than that of γ-
sanshool, as shown in Table 1. In addition, hydroxy α-
sanshool was also the weakest agonist of TRPV1 among
all the sanshool samples, although the pungent quality of
this compound was not much different from the others.
From these results it is difficult to explain the pungent
qualities of the sanshool samples simply in terms of
TRPV1 activation. Bryant and Mezine recently found
that hydroxy α-sanshool excites the cool-sensitive
neurons or acid-sensitive neurons, which are different
from the capsaicin-sensitive neurons,[10] so sanshool
compounds might activate some neurons different from
TRPV1-expressing neurons. In conclusion, we have
shown that the pungent qualities of sanshools were too
complicated to be explained solely by TRPV1, although
a part of their pungency does appear to be related to
this receptor. Further studies are required to elucidate
their action.
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References