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Exploring Natural Products for New Taste Sensations

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Abstract: This paper discusses the discovery of uncommon taste or trigeminal active compounds and their associated sensory analysis using human tasting panels with the aim of enhancing the overall taste experience whilst reducing where possible the sugar and salt content of foods. The first example outlines the discovery of the sensory quality attributes of (*R*)-2-(carboxymethylamino)propanoic acid, named (*R*)-strombine, as assessed by a panel of 47 subjects to confirm its contribution to the typical taste of scallop muscle. The second example discusses the pungency and trigeminal effect of polygodial, which is compared with piperine and capsaicin, as well as the elucidation of a new structure eliciting a trigeminal effect, (±)-*trans*-2,3,3a,7a-tetrahydro-1H-indene-4-carbaldehyde, discovered in *Amomum tsao-ko*. Finally, the time intensity trigeminal effect of (–)-menthol is compared with (1*R*,2*RS*,4*RS*)-1-isopropyl-4-methylbicyclo[3.1.0]hexan-2-ol, named dihydroumbellulol, a new cooling compound obtained by hemi-synthesis from umbellulone extracted from *Umbellularia californica* Nutt.

Keywords: (*R*)-2-(Carboxymethylamino)propanoic acid · Dihydroumbellulols · Flavor design · Polygodial · Trigeminal and taste properties

Introduction

When we eat food, our brain not only integrates the visual, touch, smell, and, to a smaller extent, noise attributes (for crunchy food), but also many other parameters relayed by specific sensors located in the mouth such as taste and trigeminal receptors.[1–9] In addition, changes in flavor over time are induced by enzymatic and bacterial processing during mastication.[10]

The ever-present innovation within the food industry means that there is a constant need to investigate natural sources for novel compounds displaying interesting trigeminal or taste effects. How can we discover these new molecules? Could the flavor industry follow the pharmaceutical industry's example by heavily investing in high-throughput screening?[11–13]

The food and flavor industry has already started to exploit this strategy, thanks to the development by certain companies of new cell-based assays with expressed G protein-coupled taste receptors for both sweet and umami tastes, commonly associated with the taste of monosodium glutamate.[3–14] This new process complements perfectly the 'classical' approach, successfully applied in our analytical laboratories over the past 100 years, which consists in the careful and focused screening of interesting animal and plant species: slow-throughput screening with a high success rate as opposed to high-throughput screening with a low success rate. This high success rate is possible only if extractions/purifications are made on selected materials having known taste or trigeminal effects. Materials are selected on the basis of information gathered from the botanical literature, ethnobotanists, personal curiosity, or field work. This approach has been very successful for the discovery of sweet natural compounds.[15,16] Screening for taste modifiers has relied on tasting of purified extracts by human subjects. Although this means very low throughput, the great advantage, compared with screening specific receptor interactions, is that we are able to assess the overall effect in the human mouth and to avoid wasting any time with false positives.

Following the isolation and characterization of novel compounds that elicit an interesting mouth effect, we undertake detailed sensory perception tests to evaluate the parameters of this effect. To do this, we require grams of material that we produce by relying either on large-scale isolation from the natural source if possible, or on the unambiguous synthesis of the active component.

Similarly, when we discover new compounds having taste modulation or trigeminal effects such as hot, pungent, cooling, or mouthfeel sensations, their sensory aspects are also assessed by a trained panel. The sensory characterization of these new compounds allows the comparison of their overall performance with benchmark market products. The sensory aspects currently assessed comprise both quantitative measures, such as detection thresholds, dose response curves, or intensity profiles over time, and qualitative measures to define the most appropriate terms to describe the product. Different types of sensory studies that we use to characterize the performance of the new compounds are presented with each example in the following sections.

The aim of this paper is to outline some of the results that we have obtained over the past few years in screening natural products to discover molecules and integrating all parameters of chemosensation to help our flavorists be innovative in their flavor design. This work was particularly motivated by the current high demand in the marketplace to reduce salt, monosodium glutamate (MSG), and/or sugar contained in highly processed foods. The holy grail of the food industry has been to find compounds that help magnify taste and the sensations that go with it.[2]

Discovery of the Importance of (*R*)-Strombine in the Taste Profile of Scallops

The special taste of some seafood such as mussels cannot be imitated by the usual food ingredients, MSG (glutamic acid (**1**) at neutral pH), salt (NaCl, KCl), sugars, or

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ribotides.[17–19] The mouthfeel of seafood is quite different from chicken broth, for example, which is fattier, saltier, and more umami. We investigated the taste-active compounds in dried scallops by bioassay guided fractionation with cation exchange chromatography, followed by liquid chromatography and multiple tastings, with the mouth being the ultimate detector. One fraction was clearly described as sweet with fullness in the mouth. After careful analysis, we detected the presence of glycine (**2**), (*S*)-alanine, and (*R*)-strombine (**3**) in this interesting fraction. (*R*)-Strombine (**3**) is known to be present in mussel muscle as a result of anaerobic metabolism. It belongs to the opines, a class of compounds resulting from the condensation of the most abundant amino acids in mussel, in this case glycine (**2**) with pyruvic acid (**4**, Fig. 1), to form a Schiff's base, which is then reduced by opine dehydrogenase.[20,21] Nothing was known in the literature about the taste properties of (*R*)-strombine. (*R*)-Strombine was prepared on a large scale *via* reductive amination of glyoxylic acid (**5**) and (*R*)-alanine (**6**)[22] to allow us to further evaluate its contribution to dried scallop taste. This compound was evaluated by an in-house panel of volunteers in accordance with our consent guidelines. Sensory characterization of these new compounds allows us to compare the robustness of their performance with benchmark products already on the market.

To assess the sensory performance of **3**, trained panelists received a 20 mL water solution in a cup (blind test). They were asked to taste the entire sample in their mouth. They spat out the sample after 5 seconds and then answered questions about it. The sensory characterization was combined with threshold measurements (ASTM E679 Ascending Concentration Series Method of Limits), in one session per product, with choices of sensory attributes. The panelists were asked to choose among sweet, salty, bitter, umami, acid, mouthfeel, and metallic or 'by chance'. Additional comments could also be added in the field 'others'.

(*R*)-Strombine is perceived mainly as salty and umami by the majority of panelists. The multisensory aspect of this compound is very interesting, especially for savory applications. The taste component of glycine is unimodal and clearly sweet from 1 g/L to 16 g/L. Unfortunately, the distribution threshold of (*R*)-strombine is centered at 0.5 g/L, which means that it is 10 times less potent than MSG[23] (Fig. 2).

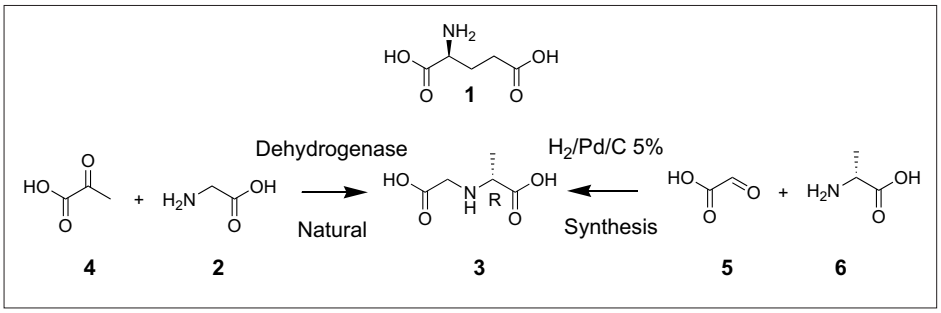

Fig. 1. Synthetic and biological formation pathways of (*R*)-strombine.

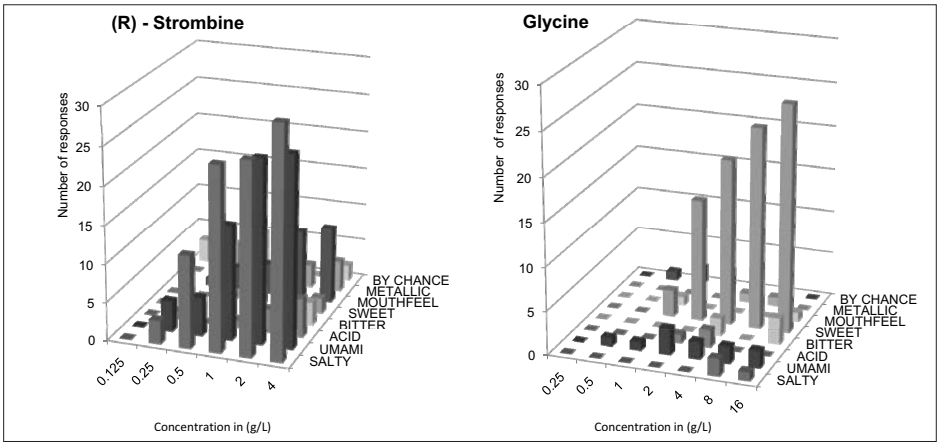

Fig. 2. Sensory attribute assessments of (*R*)-strombine and glycine, established in water solution, by 47 subjects at different concentrations.

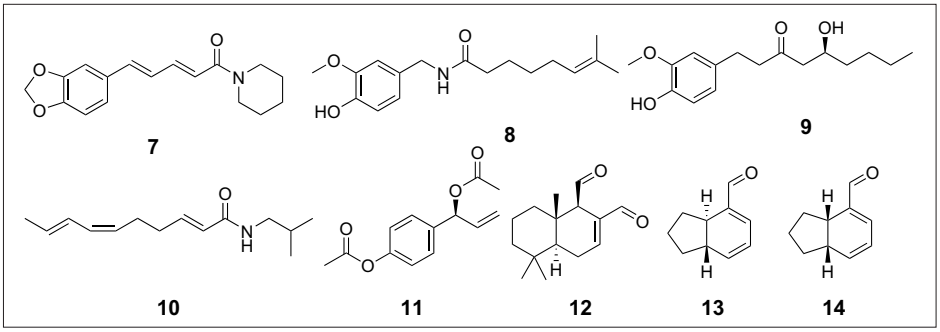

Fig. 3. Some natural molecules displaying trigeminal effects.

Investigations of the Pungent Effects of some Botanicals

The most common commercially accessible pungent molecules are piperine (**7**) from *Pipper nigrum* L.; capsaicin (**8**), the active component of chili peppers (genus *Capsicum*); and gingerol (**9**) from ginger, *Zingiber officinale* (Zingiberaceae)[24,25] (Fig. 3). Spilanthol (**10**) is a polyunsaturated amide commercially available from an oleoresin prepared from *Acmella oleracea* L. (family Asteraceae) that elicits a tingling sensation in the mouth. Spilanthol is just one example of a family of polyunsaturated amide analogues that occur in nature and display this interesting effect.[9,26,27] These molecules activate the trigeminal receptors, which are quite different from the taste receptors because they activate responses in a subset of nociceptive fibers by changing the activity of a family of temperature-sensitive ion channels that permit the entry of Na^+ and Ca^{++}.[9–13] These polymodal nociceptive receptor neurons are connected to cranial nerve V in the face, which allows us to perceive pain effects in the mouth and in the nose.

Laksa (*Persicaria odorata* (Lour.) Sojak, a coriander plant commonly used in Thai cuisine, displayed a pungency that was quite different than the pungency or tingling of **7**–**10**; it resembled the pungency of galangyl acetate (**11**) occurring in *Alpinia galangal* (family Zingiberaceae). In order to discover the active principle of laksa, which was not known in the literature, the leaves were extracted with various solvents such as water, ethyl acetate, and ethanol. Smelling strips were then dipped into these solutions and, after the solvent

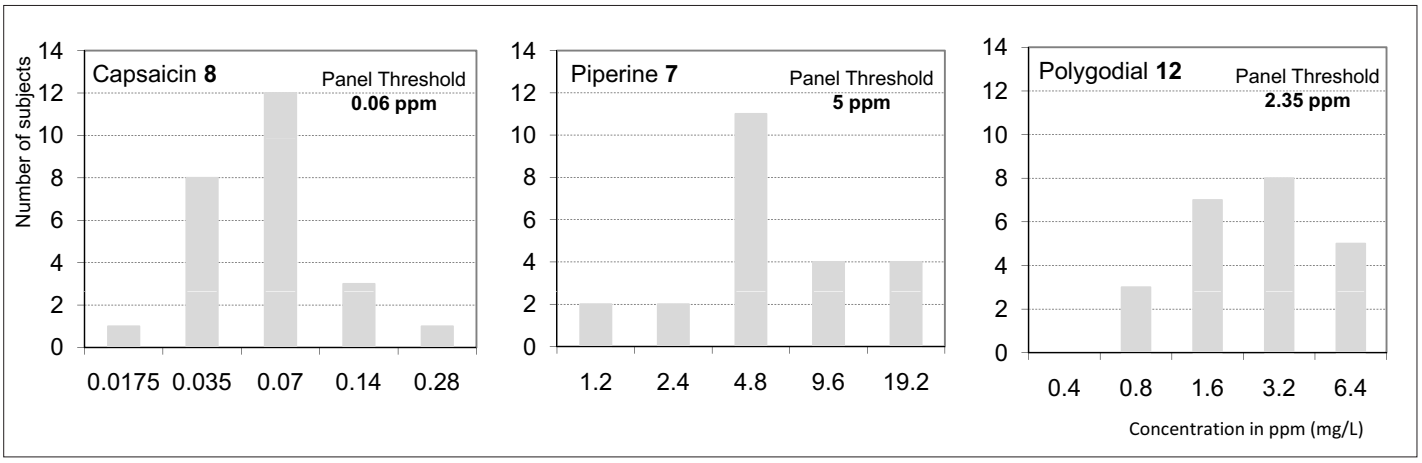

Fig. 4. Distribution thresholds for the three molecules: capsaicin (**8**), piperine (**7**), and polygodial (**12**), established by 25 subjects.

evaporated, the strips were placed on the tongue. The active compound was clearly perceived in the ethyl acetate extract. A larger quantity of leaves was extracted with ethyl acetate. After concentration of the solution, the oleoresin was purified by chromatography on silica gel and all fractions tasted to localize the pungent molecule. This led us to the discovery of the known polygodial (**12**), a compound also present in mountain pepper, *Tasmannia lanceolata* (Poir.), from the family Winteraceae.[28–30]

Polygodial (**12**) is a good example of the potential uses of pungent molecules in very different applications. It can be used, for example, in wasabi paste to enhance the pungent effect of allyl isothiocyanate without the associated rubbery olfactive note, to enhance the freshness of toothpaste,[31] or even to improve the sensory acceptability of artificial sweeteners.[32]

The trigeminal effect of polygodial (**12**), piperine (**7**), and capsaicin (**8**) was determined by a panel of 25 subjects (threshold measurements achieved with the three-alternative forced choice method based on the ASTM E679 Ascending Concentration Series Method of Limits in one session per product). According to average threshold values (Fig. 4), polygodial is twice as strong as piperine, but 40 times weaker than capsaicin. The wider distribution range for the perception of polygodial than for the other two compounds means that there are more differences between subjects for this compound.

During a taste trek in China to discover new interesting materials, we found the sensory properties of black cardamom (*Amomum tsao-ko* Crevost et Lemarié) (Zingiberaceae), widely used as a spice in China, to be refreshing because of the presence of 1,8-cineol and other known constituents. No reported constituent, however, could explain the pleasant and refreshing trigeminal effect.[33–37]

Guided by the tasting of each fraction obtained by liquid chromatography separation (LC-tasting), we found that one fraction had an unusual pungent taste. After being isolated and characterized by comprehensive spectroscopic methods, the compound present in this fraction was found to be either **13** or **14** (Fig. 3). As it was not possible to unambiguously assign the relative ring junction stereochemistry, we embarked upon the synthesis of both *cis*-**14** and *trans*-**13** isomers to confirm the structure. The synthesis employed a Wittig reaction between (*E*)-(4-methoxy-4-oxobut-2-en-1-yl)triphenylphosphonium bromide and 1-cyclopentene-1-carbaldehyde. By varying the conditions used for this Wittig reaction, we could synthesize both *cis* and *trans* ring junction isomers **13** and **14** from the same starting material.[38] Finally, by comparison of analytical data, we were able to determine that only the less thermodynamically stable *trans* compound **13**, present in black cardamom, was responsible for the trigeminal effect, described as pungent, tingling, and refreshing.[38]

Hemi-synthesis of a New Cooling Compound: (1*R*,2*R*/*S*)-1-Isopropyl-4-methylbicyclo[3.1.0]hex-3-en-2-ol

The monoterpene reported to elicit the greatest cooling effect in the literature[39,40] is (–)-menthol (**15**), and cubebol (**16**) is the only sesquiterpene reported to be cooling[41] (Fig. 5). Many synthetic compounds with diverse chemical structures have been synthesized to explore new longer lasting

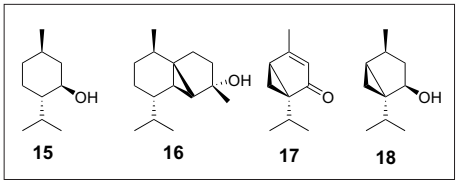

Fig. 5. Structure of some cooling compounds.

cooling effects or cooling effects without the smell of mint.[42–48]

Umbellulone (**17**) is the major monoterpene constituent present in the leaves of *Umbellularia californica* Nutt. When the leaves were crushed and smelled or tasted, a clear trigeminal effect was perceived. As a result of this interesting observation, the leaves were extracted and umbellulone isolated as the major constituent. We wished to hydrogenate the C=C double bond of umbellulone and reduce the ketone in order to compare the cooling effect of these molecules with (–)-menthol (**15**). Catalytic hydrogenation (H_2, 5% Pd/C) gave dihydroumbellulone as a mixture of isomers in good yield. This mixture of dihydroumbellulone isomers was separated and further reduced with $NaBH_4$/MeOH to give dihydroumbellulol (**18**) and its isomers. With pure **18** in hand, we wished our sensory panel to compare its cooling strength with (–)-menthol.

The intensity over time is a very important parameter, the objective of the sensory evaluations being first to confirm the cooling effect of the dihydroumbellulol (**18**), and then to compare its performance with that of (–)-menthol.[49]

Thirty trained panelists evaluated the perceived cooling intensity over time of the two samples, dihydroumbellulol and (–)-menthol (one sample per session, at 50 mg/L). Each subject received a sample (30 mL) in a cup and was asked to taste it and to start a timer at the same time. Subjects spat out the sample after 5 seconds. Panelists evaluated the time at which they started to perceive the cooling sensation (T_{begin}), the maximum perceived cooling intensity and its corresponding time (I_{max} and T_{max}), the time when the cooling sensation started to decrease (T_{dec}), and, finally, the perceived cooling intensity 3 min after they first tasted the sample (I_{end}) or the time when the cooling sensation disappeared, if it was less than 3 min (T_{end}). Each evaluation was rated on a linear scale from not

perceived (= 0) to very intense (= 10). Student *t* tests (two-tailed, paired) were performed on each parameter (T_{begin}, I_{max}, T_{max}, T_{dec}, I_{end}, and T_{end}) to compare data obtained from the two cooling compounds. The probabilities obtained for each of these tests indicate whether the compounds have been perceived as significantly different or not (significance was defined as $p < 0.05$) for the parameter under consideration. This sensory evaluation (Fig. 6) confirmed the cooling effect of **18** as having a similar type of cooling profile over time compared with (–)-menthol (**15**), but overall, it was less powerful. The I_{max} values showed that dihydroumbellulol is weaker than (–)-menthol (significant difference for I_{max}) and its persistent trigeminal effect is shorter, with a more pronounced delay.[49]

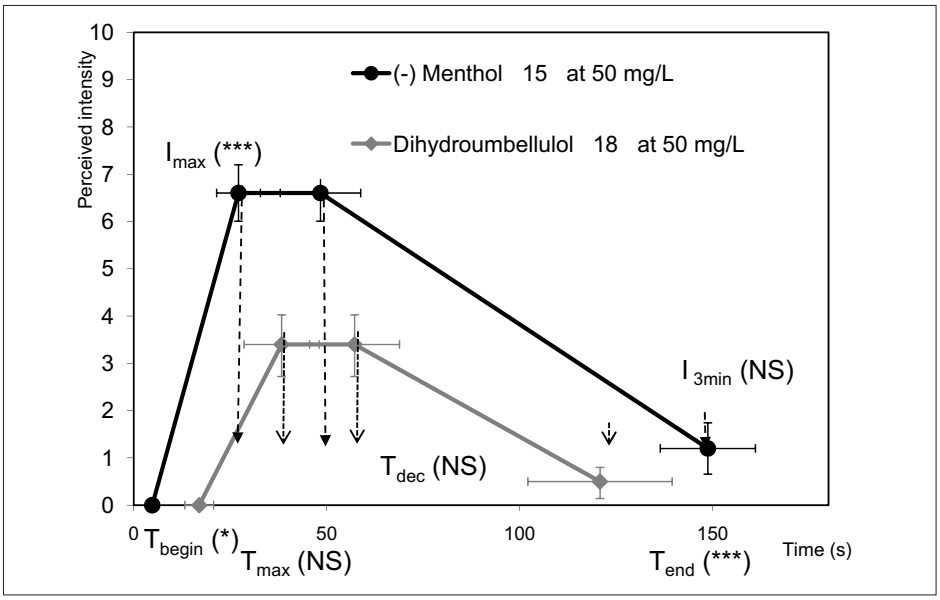

Fig. 6. Perceived intensity of cooling over time assessed by 30 subjects (panel average and confidence interval at 5%).

Conclusions

We have demonstrated that human tasting of purified compounds isolated from natural sources is a key analytical step in discovering molecules contributing to the taste of delicate foods such as seafood products. Naturally derived compounds eliciting an interesting taste or mouthfeel effect have considerable potential as innovative flavors. However, with the large volume of cheap commodity compounds such as MSG being the market benchmark, the principal hurdle becomes the cost of using these types of compounds.

In terms of actually launching commercial products, more potential can be seen in molecules producing trigeminal effects. Mouth refreshing products and saltiness and sweetness enhancers will always be molecules of interest. Our recent results have clearly shown that it is still possible to discover new molecules eliciting trigeminal effects in common spices, which motivates us to continue the search, despite this having being the subject of intense research over many years.

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