Determination of molecular structure of odor components based on crystalline sponge method

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1. Introduction

Sensing odors is an extremely important ability humans have as living organisms. This ability is required for humans to maintain appropriate eating behavior and to protect themselves from imminent danger. The term "odor" includes naturally occurring odors from food and flowers, and artificial odors from synthetic essences, as well as a variety of other smells.

Humans sense odors when they inhale airborne molecules through their nostrils. Based on this fact, odors basically consist of volatile substances (compounds). In order to learn what the molecular structure of trace amounts of volatile odor components is like, many studies and analyses have been conducted for many, many years across different technical fields.

The human sense of smell can even sniff out optical isomers. In the case of limonene's optical isomers shown in Fig. 1, whereas the d-isomer has the refreshing smell of citrus fruit, the l-isomer has the smell of petroleum. When substances with an identical composition formula but a different 3D structure are floating in the air, humans sense these substances as totally different odors. This is because sensing an odor starts with the coupling of an odor molecule with a G-protein coupled receptor (GPCR)[1]. The state of the coupling varies depending on the shape of the odor molecule, including with which GPCR the odor molecule can couple (from reportedly over 800 kinds of GPCR), at which part of the GPCR, and how tightly. It is believed that this accounts for the uniqueness of odors.

Therefore, just as the molecular structure of a pharmaceutical compound (including its absolute configuration) is important when it works as a "drug," the odor's molecular structure is considered to play a critical role when an odor is generating the intended sense of "fragrance." An extremely low concentration of an odor component can react with the receptor in the human nose in the form of odor, which means that only a trace amount of such a component exists in the environment. For this reason, if you want to extract a specific odor component from natural raw materials for analysis, you may end up obtaining only several micrograms or less from a large quantity of raw material (in the range of several kilograms to several tons). This is why gas chromatography (GC), liquid chromatography (LC), and mass spectrometry (MS) are widely used today as mainstream analytical equipment capable of analyzing extremely small amounts of samples. However, even with the help of these analytical techniques, directly determining the 3D molecular structure of odor components is impossible.

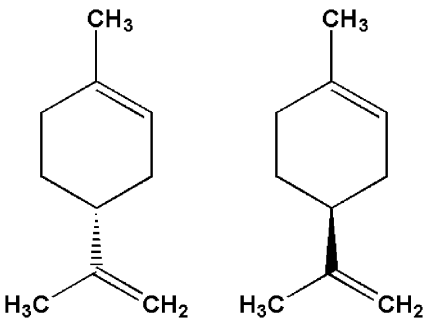

Fig. 1. Whereas the d-isomer of limonene (left) has the refreshing smell of citrus fruit, the l-isomer of limonene (right) has the smell of petroleum.

On the other hand, single crystal X-ray structure analysis is suited for the determination of the 3D structure of hitherto unknown substances. However, this analytical technique requires large quantities of high purity samples for crystallization, and for this reason it has seldom been used for the analysis of odor components in the past. The emergence of the crystalline sponge method[2] has dramatically changed this situation.

This report introduces examples of our attempts to analyze the structure of odor components, in which volatile substances emitted from natural products are directly trapped without the isolation of odor components or sample preparation, using the "crystalline sponge method"[2], a single crystal X-ray structure analysis method that does not require sample crystallization.

2. Experimental method

2.1. Concept of crystalline sponge method

The crystalline sponge method, first introduced in 2013, is a molecular structure analysis technique based on a host crystal of porous organometallic complexes called a metal-organic framework (MOF), whose cavities are filled with cyclohexane molecules. A solution containing a target compound is poured into this host crystal, and the solvent is gradually evaporated to concentrate the solution. As a result, the target compound is trapped in the cavities of the MOF. However, starting this process with a crystalline

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sponge filled with cyclohexane causes a problem. As soon as it is removed from the solvent, the crystalline sponge that contains the target compound will begin to dry on the surface, which decreases the crystallinity of the compound. For this reason, when you adopt the technique to concentrate the solution by evaporating the solvent, you need to remove the crystalline sponge while it is fully soaked in the solvent.

Additionally, the use of fully isolated/purified target compounds is recommended. This is important because if multiple guest molecules enter a single cavity of a crystalline sponge, these molecules will overlap each other in the cavity and create an irregular structure, making the analysis difficult. However, this experiment is intended to determine the structure of target compounds without extraction, separation, or purification, with the aim to trap odor components (volatile components) that are emitted from food and industrial products around us, and which we actually perceive in our everyday lives. On the other hand, in order to determine the molecular structure of unknown odor components which are floating in the air, it is necessary to trap them in an effective manner by ruling out the selection of target compounds based on their solubility in solvents. In other words, there is the need for a mechanism to expose odor components directly to the surface of the crystalline sponge.

2.2. Crystalline sponge preparation

As mentioned above, crystalline sponges have an extremely weak affinity between the MOF and solvent (cyclohexane). For this reason, if a crystalline sponge is removed from the solvent, the solvent retained in the crystalline sponge will drip, decreasing the crystallinity of the target compound. In order to expose the surface of the crystalline sponge to the atmosphere as much as possible, it is necessary to improve the stability of the crystal in the absence of the solvent. To this end, a crystalline sponge with its MOF cavities filled with trans-decalin was prepared (trans-decalin crystalline sponge). Figure 2 shows the structure of a trans-decalin crystalline sponge.

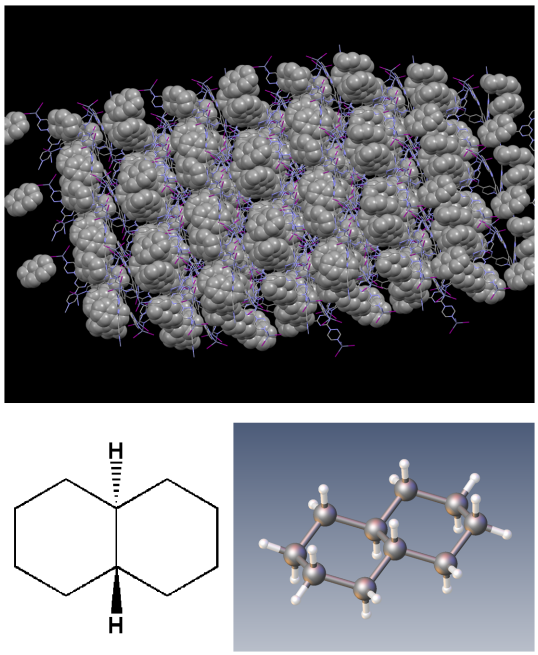

Fig. 2. Structure of trans-decalin crystalline sponge.

Trans-decalin resembles cyclohexane in geometry, and its boiling point (185℃) is higher than that of cyclohexane (80.75℃). For this reason, even when a trans-decalin crystalline sponge is removed from the solvent, it does not deteriorate. In order to trap only volatile substances, the soaking process should be conducted using this trans-decalin crystalline sponge.

2.3. Soaking procedure

In the past, the soaking process of a target compound into a crystalline sponge has been conducted by pouring a solution of the target compound into a vial containing both the crystalline sponge and cyclohexane, then gradually evaporating and concentrating the solvent (Fig. 3).

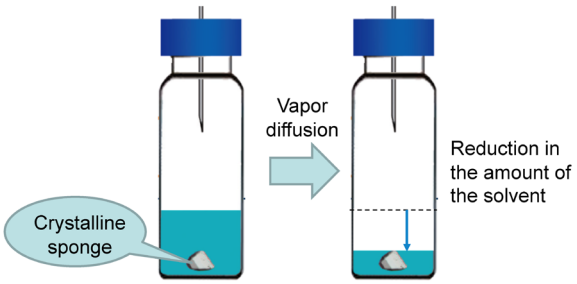

Fig. 3. Previous soaking procedure: The solvent gradually evaporates through the injection needle mounted on the cap of the vial.

In this experiment intended to trap airborne substances, the soaking process was conducted in an airtight environment (Fig. 4). This setting allows the crystalline sponge to trap only volatile components.

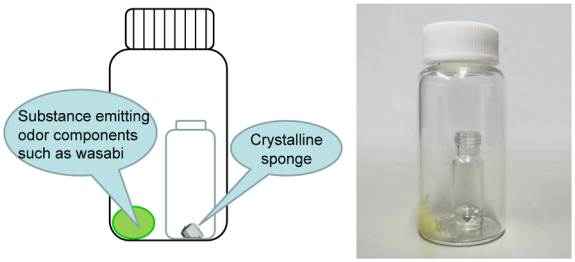

Fig. 4. Soaking procedure for this experiment.

The amount of target compound a crystalline sponge will trap is approximately proportional to the soaking time. When trapping a target compound that discolors the crystalline sponge, the color of the crystal itself will gradually change over time (Fig. 5). However, when the soaking process continues for a long time, the

crystallinity frequently decreases due to the vaporization of trans-decalin, among other reasons. As a result, soaking time was limited to a maximum of about three days.

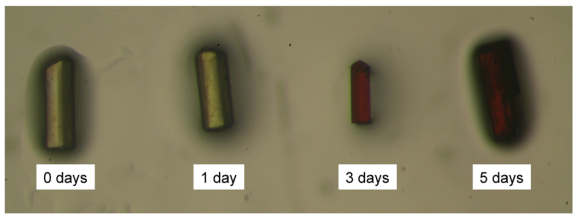

Fig. 5. Changes in crystalline sponge color with soaking time when trapping cypress essential oil.

3. Analysis results

3.1. Fresh wasabi

Commercially available fresh wasabi was analyzed using the crystalline sponge method. The sample was soaked for about a day using the method described in section 2.3., after which it underwent structural analysis. The molecular structure of allyl isosulfocyanate could be determined (Fig. 6). In this experiment, the electron density peak of the sulfur atom contained in the molecule was clearly observed, by which the position of the molecule in a cavity could be easily identified.

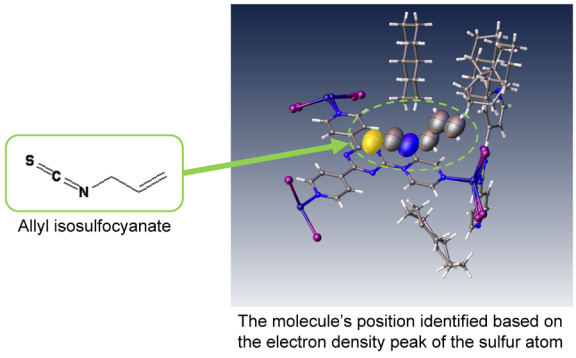

Fig. 6. Analysis results of fresh wasabi: The structural formula of allyl isosulfocyanate (left) and the 3D structure of the allyl isosulfocyanate molecule trapped by the crystalline sponge (circled in green in the right figure).

3.2. Fresh garlic

Commercially available fresh garlic was analyzed using the crystalline sponge method. The sample was soaked in the same way as the fresh wasabi, and Fig. 7 shows the analysis results.

In this experiment, the structure of a diallyl sulfide molecule could be determined. In addition to the electron density peak identified as the sulfur atom of a diallyl sulfide molecule, multiple electron density peaks considered to be derived from sulfur atoms were observed. However, diallyl sulfide was the only molecule that was analyzed in perfect condition. The identity of other electron density peaks could not be understood.

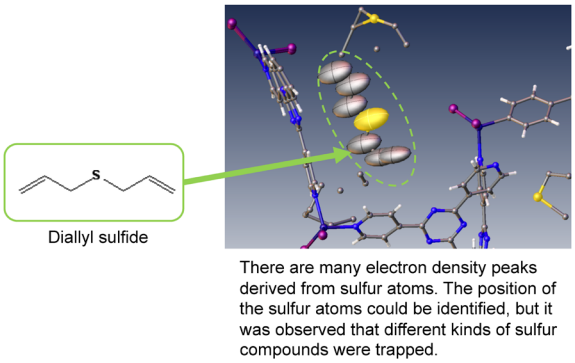

Fig. 7. Analysis results of fresh garlic: The structural formula of diallyl sulfide (left) and 3D structure of the diallyl sulfide molecule trapped by the crystalline sponge (circled in green in the right figure).

3.3. Dishwashing detergents

Commercially available dishwashing detergents contain various kinds of essences. In this experiment, a dishwashing detergent with lime fragrance was selected as the sample for structural analysis (Fig. 8).

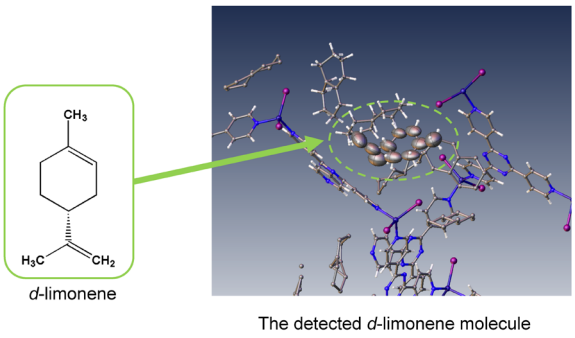

Fig. 8. Analysis results of the dishwashing detergent: The structural formula of *d*-limonene (left) and the 3D structure of the *d*-limonene molecule trapped by the crystalline sponge (circled in green in the right figure).

Generally, lime essences are mixtures of multiple essences, and *d*-limonene was clearly identified in this analysis. When molecules that are easy to trap and molecules that are difficult to trap coexist, crystalline sponges preferentially trap the former molecules. Limonene has a higher affinity with the crystalline sponge than other molecules, trapping it preferentially by the crystalline sponge. This fact is considered the reason why only limonene was identified in this experiment.

3.4. Cypress essential oil

Generally, cypress essential oil contains a large amount of pinene. In this experiment, α-pinene was detected after the samples were statically soaked for about a day (Fig. 9).

However, when the soaking time was extended to

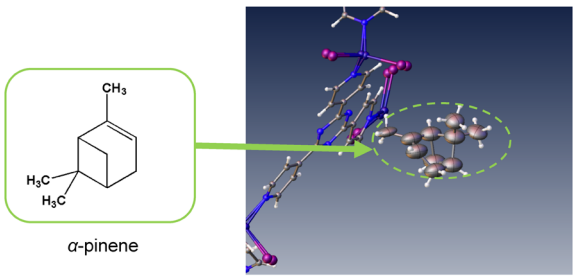

Fig. 9. Analysis results of cypress essential oil (after being soaked for a day).

three days or more, it was no longer possible to identify pinene, let alone molecules other than pinene. This is probably because multiple molecules other than pinene were trapped in the crystalline sponge, which produced too irregular a structure to model.

3.5. Peppermint oil

In this experiment, two types of commercially available peppermint oil from different makers were analyzed. A wide variety of peppermint oils are available in the market today, including those whose main component is a synthetic essence, as well as those blended with natural peppermint. Peppermint oil whose main component is a synthetic essence was analyzed first in this experiment.

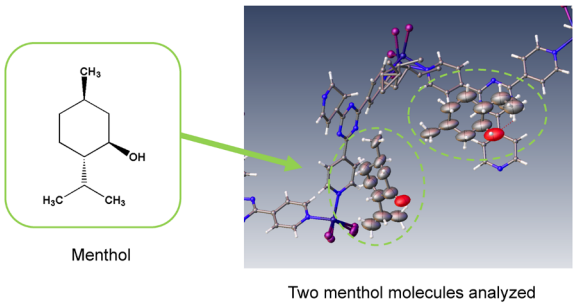

Fig. 10. Analysis results of commercially available peppermint oil whose main component is a synthetic essence: The structural formula of L-menthol (left) and its molecular structure (circled in green in the right figure). Two menthol molecules are trapped in a cavity of the crystalline sponge.

In the experiment using peppermint oil whose main component is a synthetic essence, menthol molecules were trapped and their structure was determined (Fig. 10). This is probably because the crystalline sponge preferentially trapped the large amount of menthol contained in the peppermint oil as its main component. On the other hand, carvone and menthol were detected in the commercially available peppermint oil whose main component is natural peppermint oil. There are many kinds of natural peppermint oil, including Japanese peppermint, peppermint, and spearmint. The main component of Japanese peppermint is menthol, and the main component of spearmint is carvone. From the fact that menthol and carvone were identified in the natural peppermint oil used in this experiment, it can be inferred that this product is a blend of at least two kinds of natural peppermint oil: Japanese peppermint and spearmint (Fig. 11).

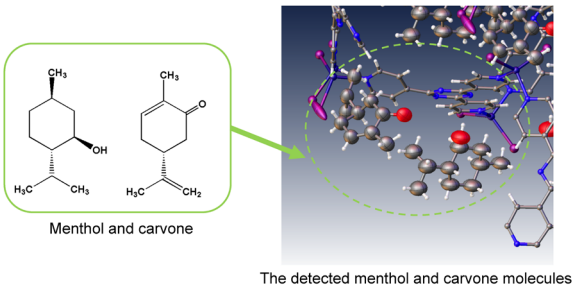

Fig. 11. Analysis results of commercially available peppermint oil whose main component is natural peppermint oil: The structural formula of menthol and carvone (left) and their 3D molecular structures (circled in green in the right figure). Both molecules are trapped in a cavity of the crystalline sponge.

4. Conclusion

The crystalline sponge method is a powerful technique that allows single crystal X-ray structure analysis to be applied even to target compounds that are difficult to crystallize. This method was very useful in determining the structure of volatile odor components, because it could be applied to small amounts of target compounds, on the order of nanograms. In this experiment, each volatile substance, which contains multiple components, was applied directly to the crystalline sponge, without separation, and the 3D structure of the trapped molecules was determined by single-crystal X-ray structure analysis. In this setting, only the structure of those molecules preferentially trapped in the crystalline sponge could be determined. If applied to each target component that was isolated and purified, and in combination with GC, LC, and MS, this method will enable molecular structure determination for extremely small amounts of target components. This type of analytical method had been entirely impossible before the emergence of the crystalline sponge method.

References

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(2) Y. Inokuma, S. Yoshioka, J. Ariyoshi, T. Arai, Y. Hitora, K. Takeda, S. Matsunaga, K. Rissanen and M. Fujita: *Nature*, **495** (2013), 461–466.