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Separation of Odor Constituents by Microscale Fractional Bulb-To-Bulb Distillation

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1. Introduction

An important task in natural products chemistry is finding new compounds with novel properties and structures. Moreover, in fragrance chemistry, the odors of constituents are evaluated and key compounds are identified that contribute to the scent profiles of fragrance materials. Figure 1 shows the general investigative process for achieving these goals of fragrance chemistry.

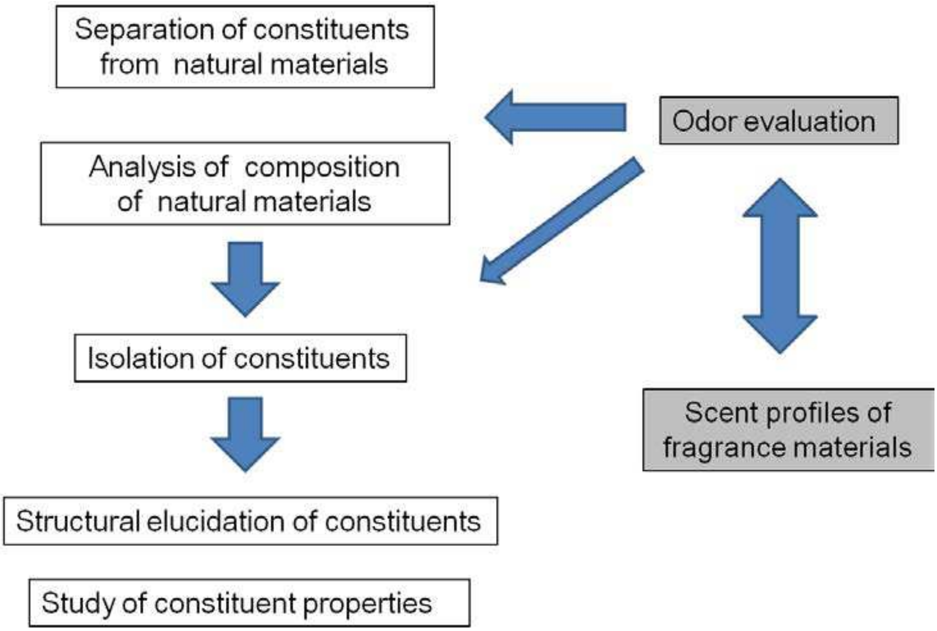

Fig. 1. General investigative process for identifying new odor compounds with novel properties and structures in fragrance organic chemistry.

Extraction of odor plants gives oils containing many compounds. A popular and useful method for analyzing the composition is gas chromatography/mass spectrometry (GC-MS), which provides important information about the scent profiles of fragrant plants. The analytical advances in the latest GC-MS technology are remarkable. In testing for the presence of a particular compound, detection of trace amounts of the substance has become possible. Recently, the olfactometry (O) functionality was incorporated into the GC-MS method. That analytical method, GC-MS-O, enables the odors of each constituent in a mixture to be precisely evaluated. These advances in analytical technology are associated with the discovery of natural products with important properties, for instance, a unique flavor. However, because the level of analysis is extremely detailed, the number of components in the characteristic scent of a plant can become huge. For this reason, indentifying the important scent components out of the many components is nontrivial. As a solution, a method for measuring the relative strength of each constituent's scent has recently been developed, and key findings have been reported. However, this method has the following problems.

1. As the number of components increases, the effort required for analysis becomes greater.
2. In a mixture of multiple components with similarly strong scents, the essential components are difficult to identify.

Such difficulties arise in elucidating the key fragrance constituents of essential oils obtained from fragrance materials, such as sandalwood and vetiver, which are currently used as base notes.

We found that the fragrances of these scent materials can be expressed by combining groups of scent components. These groups consist of several organic compounds that have similar structures and thus similar odor. The interaction between these constituents is vital to the odor. From an oil, it is important to separate the *minimum odorant groups* necessary to retain the characteristic fragrance of the scent material.

In this chapter, I will explain how to divide the odor oils from fragrant plants into groups with similar properties (structure, odor, etc.). I will also present the results of experiments conducted in my laboratory, which used popular and important scent materials.

Sandalwood (*Santalum album,* L.), vetiver (*Vetiveria zizanioides*), patchouli (*Pogostemon patchouli*), and frankincense (*Boswellia papyrifera*) are typical materials in traditional Japanese incense, and the essential oils obtained from these materials possess unique and valuable features such as providing the base notes for perfume. Although many studies on the constituents of these materials have been reported, the key compounds of their scent profiles remain unknown. Determining these scent profiles is a prerequisite to elucidating the key components of the characteristic odors of these materials.

In attempting such a determination, however, the following main issues must be overcome. Firstly, the fragrances of the scent materials are not formed by a mere superposition of individual scents. Secondly, the scent components of these materials have weak odors. Almost all researchers have recognized these issues, but previously developed methods have not been suitable for clarifying the odor characteristics. In our work, we reconsidered and revised previous methods and were able to successfully evaluate the scent profiles of these materials

2. Microscale fractional bulb-to-bulb distillation

2.1 General procedure of microscale fractional bulb-to-bulb distillation

Commercially available, a bulb-to-bulb distillation apparatus (see Fig. 2) is usually used to purify a small amount of organic material (10-500 mg) by distillation under vacuum. Glass bulbs of about 3-3.5 cm in diameter are connected in series. The joint between bulbs, for example, between bulb A and bulb B, is made of ground glass. The general procedure for performing a distillation on this apparatus is given below.

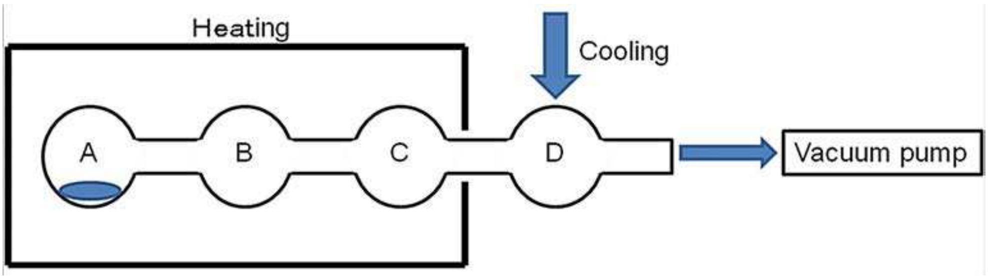

Fig. 2. Sketch of bulb-to-bulb distillation apparatus.

1. Oily sample is placed in bulb A.
2. The apparatus is evacuated.
3. Bulbs A, B, and C are placed into an oven and heated slowly. While the other bulbs are being heated, bulb D is placed in a cooling bath (e.g., ice or water).
4. The first fraction is collected from bulb D.
5. Next, bulb C is removed from the oven and becomes a collection bulb. After cooling to room temperature, bulb C, along with bulb D, is placed in a cooling bath. Then, bulbs A and B are heated.
6. Finally, bulb A is heated and bulbs B, C, and D are cooled.

Cooling all the bulbs outside the oven is a critical part of this procedure. Failure to do so will result in low recovery and poor separation.

Using this method, we can perform separations on small amounts of material (down to 10 mg) to obtain fractions with different boiling points.

2.2 Separation of odor constituents of representative incense by bulb-to-bulb distillation technique

We used fractional bulb-to-bulb distillation to evaluate the odors of oils obtained from incense materials (sandalwood, frankincense, etc.). We extracted the essential oils of the incense first with hexane and then with methanol. We compared the odors of the hexane-extracted essential oils to the original base materials. We found that the odors of the extracted oils were similar to the odors of the base materials. We performed the following fractional distillations to obtain the minimum odorant groups constituting the fragrances of the scented materials (Fig. 3). We found that the fragrances of these scent materials could be expressed by combining these groups. Each group consists of several organic compounds with similar structures and thus similar odors.

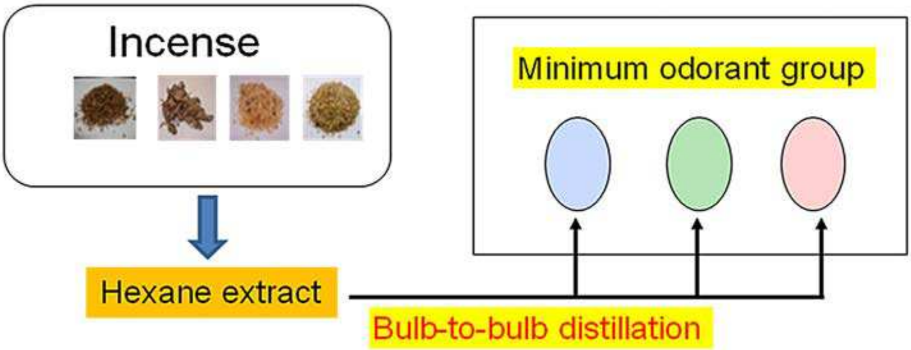

Fig. 3. General procedure for separating constituents from extract by microscale fractional bulb-to-bulb distillation.

2.2.1 Sandalwood

High-quality sandalwood—from which the essential oil is collected—is a valuable and expensive material because it can only be obtained from mature sandalwood trees. Sandalwood is a medium-sized evergreen parasitic tree and is found in India, Malaysia, and Australia. The highest quality of Sandalwood trees for incense and perfume are grown in India (especially East India). Many investigations on the composition of sandalwood essential oils have been carried out, and more than 300 constituents have been identified. The main constituents are α-santalol and β-santalol. These compounds have distinctive woody odors. Many studies have been done on sandalwood, and the structure–odor relationships of β-santalol and its related compounds have been investigated in detail.

Recently, we reported that the odor of sandalwood chips is formed by a combination of santalols and their aldehyde and formate derivatives (Hasegawa et al., 2011). Here, we will examine the interesting relationship between the structure and odor of α-santalol and its derivatives having modified side chains. Recently, we identified new odor constituents of sandalwood by the method shown in Fig. 4

We applied the distillation method introduced in this chapter to the evaluation of sandalwood odor.

We collected the hexane extract and the steam-distilled oil from sandalwood chips and compared their odors with the odor of sandalwood chips. The odor of the extracted oil was found to be similar to the odor of the base material.

The ^{1}H NMR spectroscopy revealed that the main constituents were α-santalol and β-santalol with an extremely small amount of compounds with a formyl group (Fig. 5).

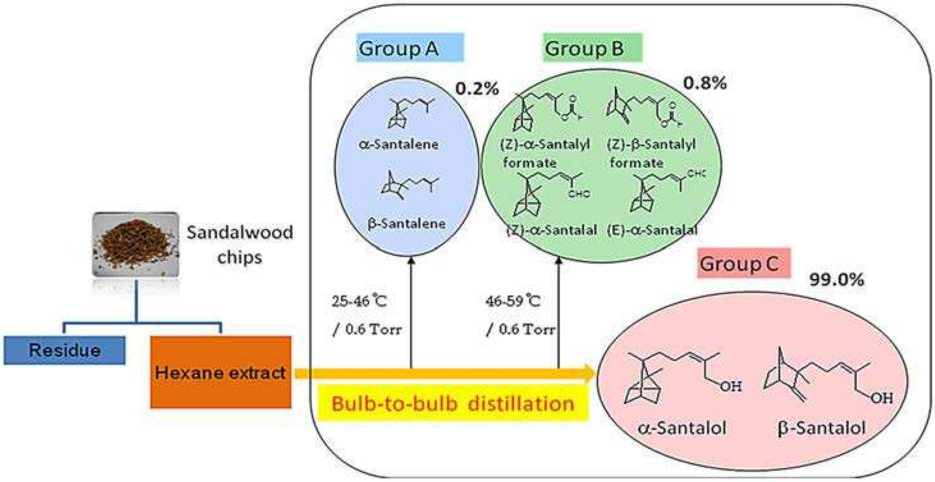

Fig. 4. Separation of odor constituents from hexane extract of sandalwood chips.

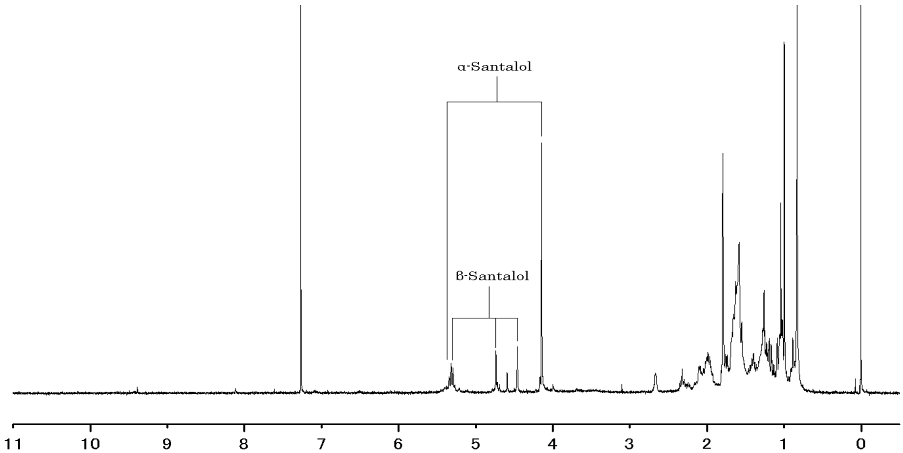

Fig. 5. ^{1}H NMR Spectrum of the hexane extract of sandalwood chips (200 MHz, $CDCl_3$).

Generally, compounds with a formyl group (aldehyde and formate) are important odor constituents. These compounds, especially aldehydes, are common decomposition products of the corresponding carboxylic acids. Aldehydes, because they are prone to decomposition, are difficult to collect from an extract by chromatography or distillation. The bulb-to-bulb distillation method, however, is suitable for handling these compounds, because the heating time is shorter than that in a typical distillation.

We performed bulb-to-bulb distillation of the hexane extract. Two fractions were obtained, and the residue was composed of α-santalol and β-santalol. The first fraction was a mixture of santalol hydrocarbon derivatives and the second fraction was santalyl aldehydes and formates, as determined by NMR spectroscopy (Fig. 6).

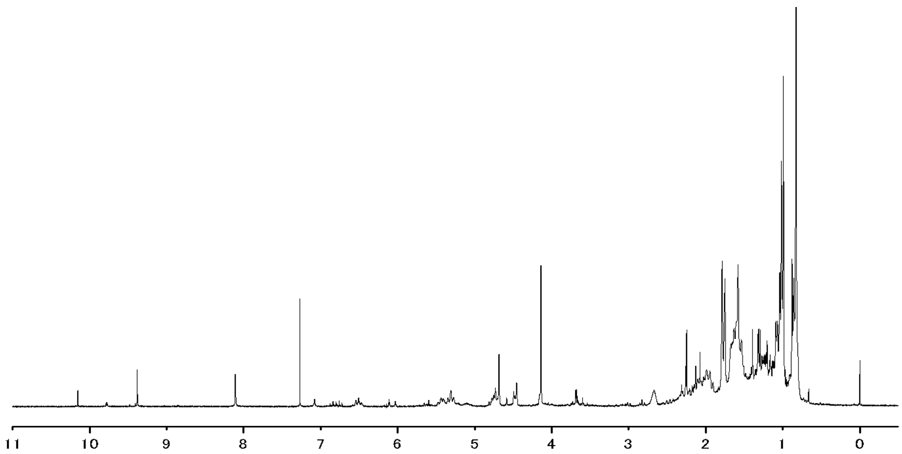

Fig. 6. ^{1}H NMR spectrum of group B obtained from hexane extract by bulb-to-bulb distillation (200 MHz, $CDCl_3$).

The obtained fractions were analyzed, and santalol derivatives with a formyl group (Group B) were found to play an important role in the odor of sandalwood chips. The diagram (Fig. 4) indicates the scent profile of sandalwood obtained by this method. β-Santaol has been reported to be the principal constituent of sandalwood odor. In contrast, α-santalol has been reported to be only a supporting component of sandalwood odor because α-santalol has a weaker odor than does β-santalol. However, we found that both α-santalol and β-santalol derivatives with a formyl group were important constituents of sandalwood odor.

This result demonstrates that bulb-to-bulb distillation is useful for collecting very small fractions from a mixture.

2.2.2 Patchouli

The unique woody aroma of patchouli is one of the four major woody notes derived from essential oils, and serves as an indispensable scent in modern fragrance. Although many studies have been performed, the key components that constitute this odor have not been successfully identified (Nabeta et al., 1993; Singh et al., 2002). Suitably appraising patchouli fragrance is crucial in order to produce potentially useful synthetic compounds.

Despite being a topic of investigation for many years, the complete odor profile of patchouli remains elusive for three reasons: first, the scents of individual compounds are weak; second, the compounds are structurally diverse and complex; and third, the aroma changes over time. To overcome these obstacles, we performed bulb-to-bulb distillation of the patchouli hexane extract, which had a similar odor as the base material.

The composition of the hexane extract of patchouli leaves was analyzed by ^{1}H and ^{13}C NMR spectroscopy. One constituent of the extract was found to be patchoulol, but its content was low and the other constituents were unidentified. We presumed that the extract contained a large amount of odorless constituents and thus attempted to collect only odor constituents from the hexane extract. Bulb-to-bulb distillation of the hexane extract produced two groups (group A and B) with characteristic odors; the residue did not have a significant odor.

Group A consisted of several sesquiterpenes and anethole (Fig. 7). Group B consisted of almost entirely patchoulol. These two groups were found to contain the key compounds that contribute to patchouli odor.

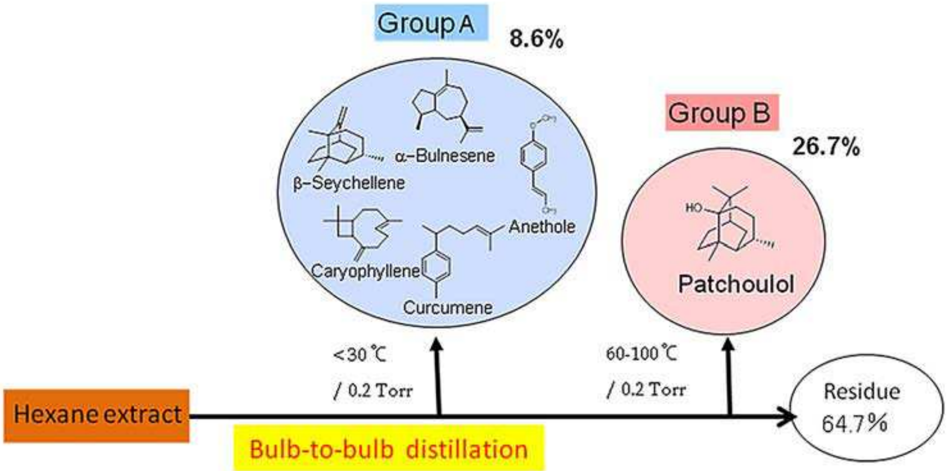

Fig. 7. Separation of scent components from hexane extract from patchouli leaves.

2.2.3 Frankincense

The resin of frankincense is obtained from many species of trees in the genus Boswellia. Frankincense has been used as a valuable fragrance source since ancient times, and has been reported to possess a wide range of bioactivity. Many compounds have been identified in frankincense (Hamm et al., 2005; Mertens et al., 2009). To our knowledge, however, the effects of particular odor components have not been clarified in detail. There are two representative species of frankincense. The main components of frankincense are markedly different between these two species. One has many monoterpenes (e.g., α-pinene) as key compounds that contribute to frankincense odor. The other contains diterpenes as the main constituents, along with octyl acetate and octanol. This latter species is used in traditional Japanese incense.

The hexane extract of frankincense is a highly viscous oil, suggesting that it contains a large amount of compounds that contribute relatively little to the characteristic odor of frankincense. The NMR spectrum of the extract (Fig. 8) supports this assessment. We did a bulb-to-bulb distillation to evaluate the key compounds of frankincense odor. First, fraction 1 was obtained from distillation below 124 °C at 0.09 Torr. The constituents were octanol and octyl acetate (Fig. 9). Then, the temperature and pressure were maintained at 124 °C (0.09 Torr), and highly similar constituents were collected in the three different bulbs according to the slightly different boiling point of each constituent (Fig. 9).

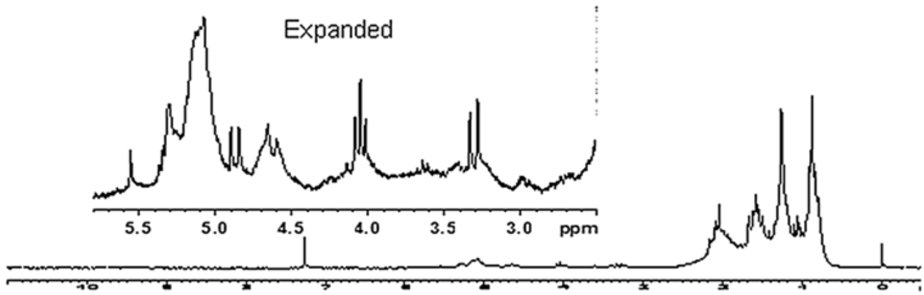

Fig. 8. ^{1}H NMR spectrum of hexane extract from frankincense resin (200 MHz, $CDCl_3$)

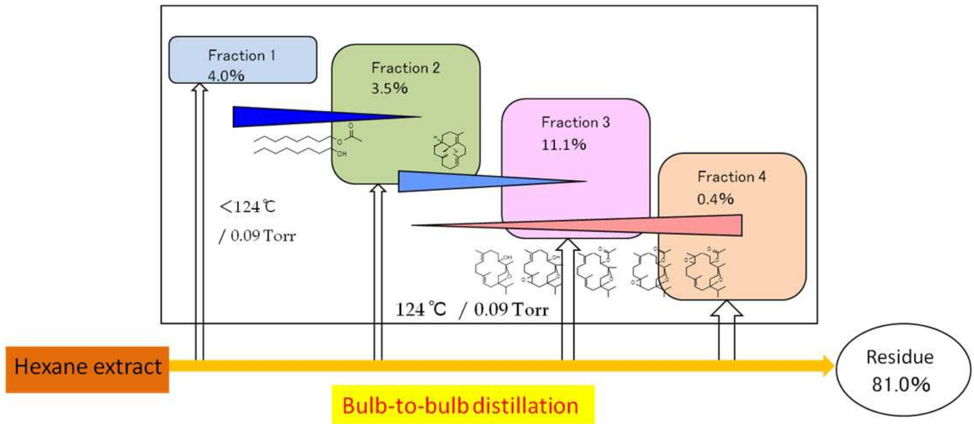

Fig. 9. Separation of scent components from the hexane extract of frankincense resin.

We evaluated the odor of this species by focusing on the difference in odor between the hexane extract and the steam-distilled oil and separation of odor components by bulb-to-bulb distillation.

NMR revealed that each fraction contained different components (Fig. 10). The odor of fraction 4 was similar to that of the hexane extract. The main components of fraction 4 were found to be diterpenes—in particular, incensole derivatives. This result shows that these incensoles make a key contribution to the odor of frankincense (Hasegawa et al., 2011).

2.2.4 Vetiver

Vetiver essential oil is a spice that provides base notes, similarly to materials such as sandalwood and patchouli. Vetiver is used as an essential material for providing fragrance, for instance in perfume, that emerges comparatively late and lasts a long time. Vetiver is said to provide the heart of the perfume. Although vetiver is important in terms of fragrance, there is still much research to do into its odor properties.

Conventionally GC-MS has been used to analyze scent components (Anonis, 2004; Weyerstahl et al., 2000), but we thought that this method would be insufficient for

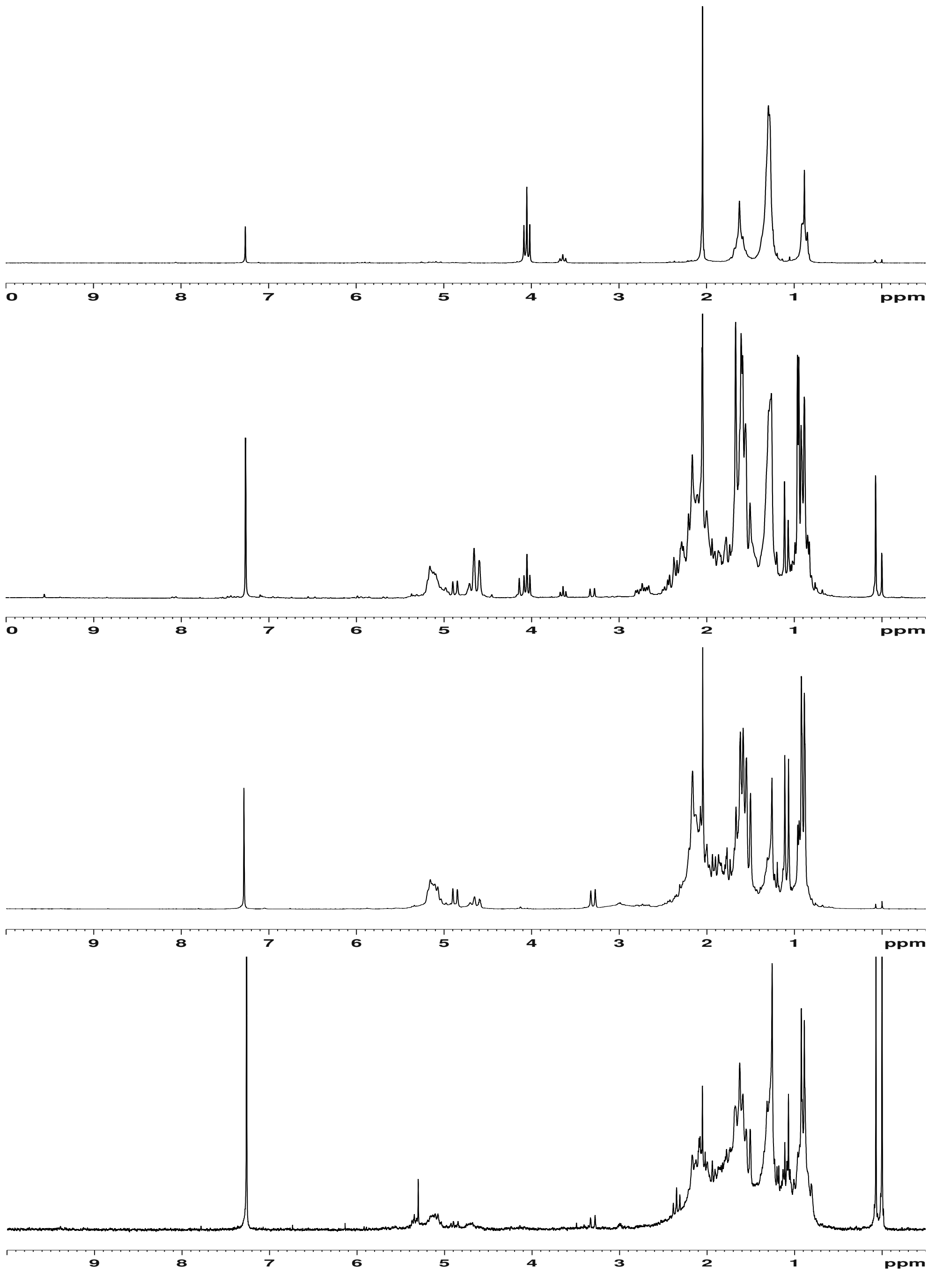

Fig. 10. 1H NMR spectra of fractions 1, 2, 3, and 4 from top to bottom (200 MHz, $CDCl_3$)

identifying the complex combinations of odorants that form the overall vetiver scent. Then, we used bulb-to-bulb diffraction to divide the hexane extract into groups with different characteristic odors; each group contained structurally similar compounds (Fig. 11).

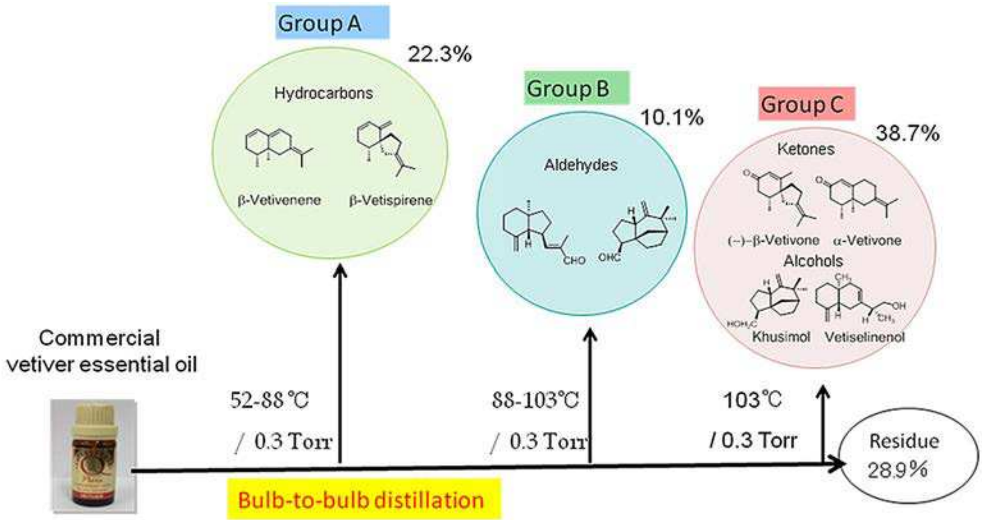

Fig. 11. Separation of scent components from commercial vetiver essential oil.

The ¹H NMR spectrum of a commercial vetiver essential oil (Quinessence Aromatherapy Ltd.) indicates that this oil was constituted by many compounds (Fig. 12). The separation of groups with different odors and different structures could not be accomplished by the aforementioned distillation procedure. In this case, the lower boiling group, group A, was first separated from the oil; then the distillation was stopped and bulbs B and C were replaced with fresh ones. We succeeded in the separation of the oil (Fig. 13). If this change was not done, the separation was poor.

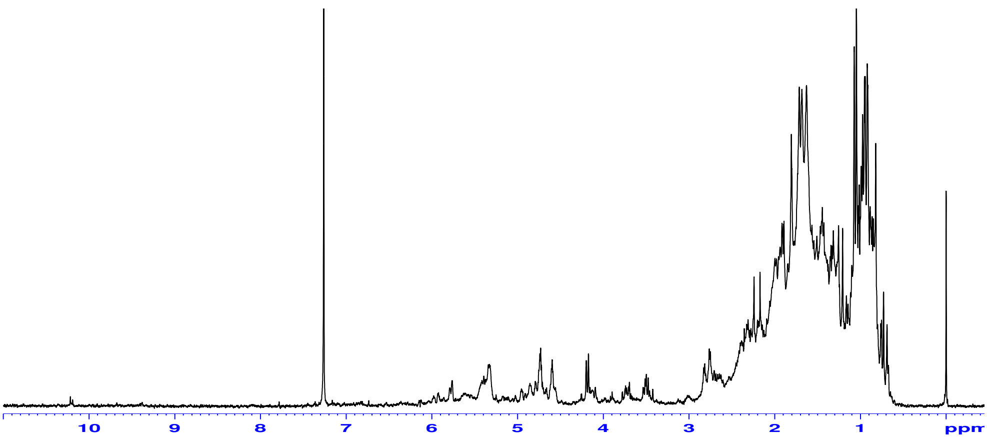

Fig. 12. ¹H NMR spectrum of commercial vetiver essential oil (200 MHz, $CDCl_3$).

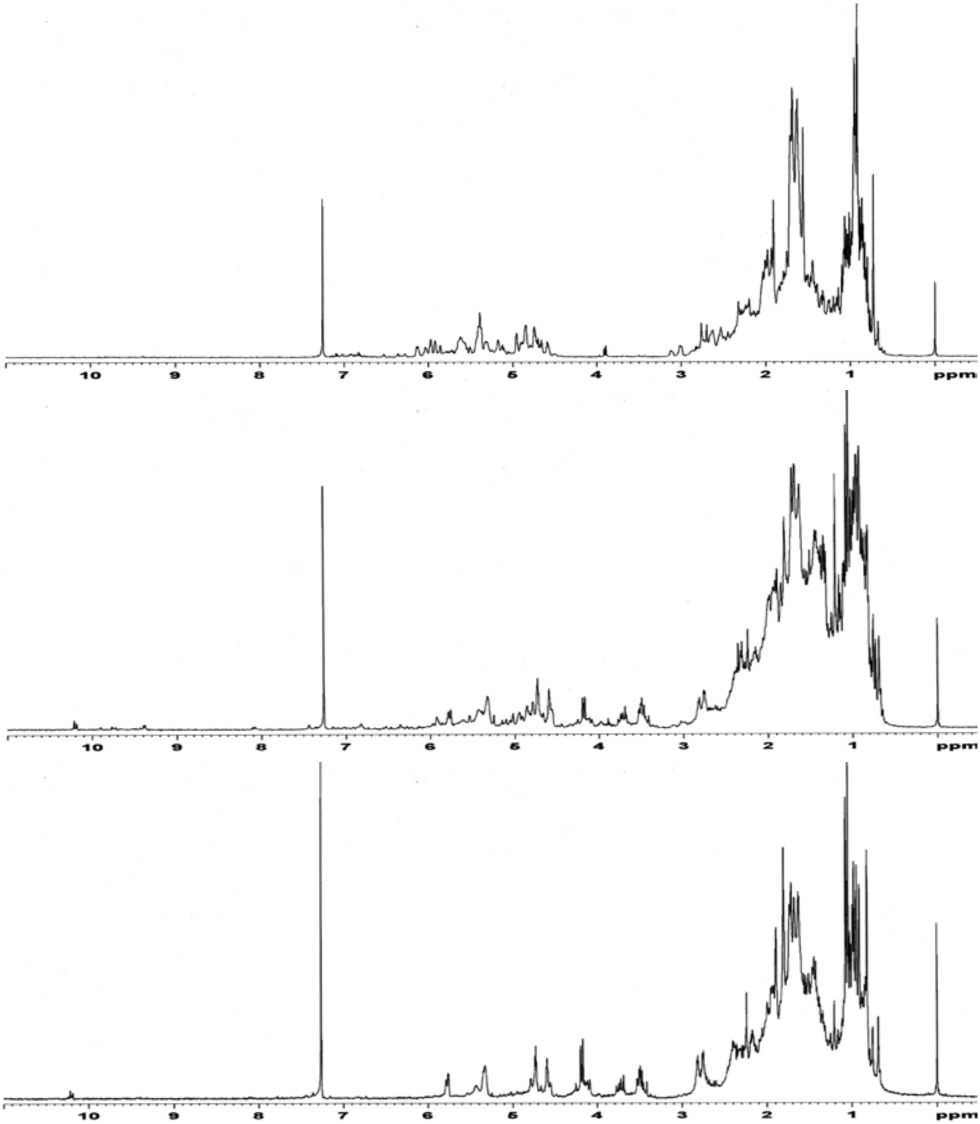

Fig. 13. ^{1}H NMR spectrum of Group A, B, and C from top to bottom (200 MHz, $CDCl_3$)

3. Conclusion

In the field of fragrance chemistry, the determination of scent profiles is a fundamental and vital endeavor. However, almost all essential oils that are used as fragrances are composed of many types of odorants, and the fragrances of the scent materials are not formed by mere superposition of individual odorants. This problem has hindered the precise evaluation of the base constituents of fragrance. We have demonstrated that the complex scent profiles of materials can be clarified by microscale fractional bulb-to-bulb distillation. To determine important components in natural materials with complex compositions, the method presented in this chapter is useful not only for perfumery chemicals but for many substances in natural products chemistry.

4. Acknowledgments

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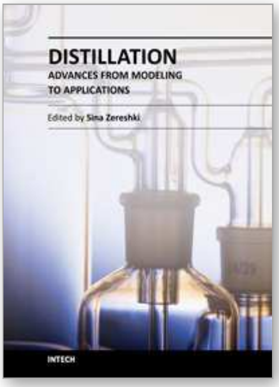

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Distillation modeling and several applications mostly in food processing field are discussed under three sections in the present book. The provided modeling chapters aimed both the thermodynamic mathematical fundamentals and the simulation of distillation process. The practical experiences and case studies involve mainly the food and beverage industry and odor and aroma extraction. This book could certainly give the interested researchers in distillation field a useful insight.