Deodorizing Effect of Japanese Cypress (<i>Chamaecyparis obtusa</i>) Sawdust and Monoterpenes on Trimethylamine

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The sawdust of Japanese cypress (<i>Chamaecyparis obtusa</i>) and the monoterpenes limonene, α-pinene and β-pinene, had a deodorizing effect on trimethylamine (TMA). Trimethylamine concentration decreased as the amount of sawdust increased, indicating that the sawdust produced in a sawmill is useful to deodorize fishy odors. The GC-MS analysis determined that the volatile monoterpenes, limonene, α-pinene and β-pinene, are components of the essential oils of Japanese cypress. The monoterpenes also possessed a deodorizing effect on trimethylamine, with β-pinene being the most effective. The deodorizing mechanism was investigated through NMR and electron charge calculations for the monoterpenes. The NMR results showed that every monoterpene interacted weakly with trimethylamine. The protons with the strongest downfield shift were the monoterpene double-bond protons. Calculations of the electrical charges of monoterpenes showed that the double-bond protons had a larger positive charge than those of the other protons. Consequently, the positive charge of the monoterpene attracts the negative charge of trimethylamine, resulting in changes in the electric charge and/or small conformation changes in the monoterpenes. This appears to be the deodorant mechanism of monoterpenes on trimethylamine.

1. Introduction

Trimethylamine (TMA) is a malodorous material generated from fishy waste and manure<sup>3</sup> and is regulated in Japan<sup>9</sup>. However, no effective method for deodorizing TMA is available; therefore, the manure and feed industries cannot use a large quantity of fish. Generally, amine odors can be suppressed with acids, such as acetic acid, but this treatment may reduce the durability of the container material<sup>3</sup>. The odor of amines such as ammonia can be removed by adsorption onto activated carbon<sup>3</sup> and microporous zeolite<sup>9</sup> and by chemical reactions with tea catechins<sup>8</sup> and pyrroligneous liquids<sup>5,7</sup>. However, the industrial use of these methods is not practical because of cost.

Some herbs, such as laurel and sage, deodorized TMA, but a detailed analysis of the chemical compound(s) in their herbs responsible for the deodorizing action was not performed<sup>9</sup>. The deodorizing effect of the monoterpenes limonene, α-pinene and β-pinene and the essential oil extracted from pine needles on ammonia has been reported, but effects on TMA were not investigated<sup>9</sup>. The abundant sawdust produced in sawmills is considered waste, except for some use as fuel. Sawdust from the Japanese cypress (<i>Chamaecyparis obtusa</i>) is a readily available and inexpensive material in Japan. Various terpenes are contained in the essential oil of conifers such as pine and cypress, and their use for relaxation and as an insecticide have been reported<sup>10,11</sup>.

The present study describes the deodorizing effect of Japanese cypress sawdust and monoterpenes on TMA. The monoterpenes limonene, α-pinene, and β-pinene were used as reference compounds for the component analysis of the essential oil extracted from Japanese cypress. Analysis of the TMA deodorizing mechanism was conducted using NMR and computational chemistry analysis.
2. Materials and Methods

2.1 Materials

Limonene and terpinyl acetate were purchased from Sigma (St. Louis, MO, USA) and Santa Cruz Biotechnology (Dallas, TX, USA), respectively. The TMA solution (30%), α-pinene, β-pinene, α-terpineol, and bornyl acetate were purchased from Wako Pure Chemical Industries (Osaka, Japan). The sawdust (particle size distributions: \(D_{10}, D_{50}, D_{90} = 28, 750, 1700 \mu m\), moisture content: 43%)\(^{12}\) and the essential oil of Japanese cypress were supplied by Katsura lumber group (Wakayama, Japan).

2.2 TMA Deodorizing experiment

A 6 L glass desiccator was used for gas-phase adsorption experiments (Fig. 1). Predetermined amount of sawdust was collected on a glass petri dish and sealed in the desiccator. 3 µL of 30% TMA solution and 3 µL of terpene were collected with a separate microsyringe and injected into a glass syringe. After loading the glass syringe with a piston, TMA and terpene were evaporated by heating with a hair dryer and injected into the desiccator, perfectly. TMA concentration in the gas phase was measured using a gas collector (Gastec GV-100S) with a gas detector tube (Gastec No. 180 or 180L). TMA concentration was monitored 3 times over 3 hours (Fig. 2). A corrected value was calculated using the equation shown below, which takes into account the dilution of TMA at every sampling by the gas collector.

\[
c = c' \cdot f_T \cdot V/V_0
\]

- \(c\): corrected TMA concentration
- \(c'\): measured TMA concentration
- \(f_T\): factor of temperature correction
- \(V\): decreased volume upon sampling by the gas collector
- \(V_0\): apparatus volume

During the deodorizing experiment, the sawdust was placed in the apparatus before the TMA solution (3 µL) was vaporized. The sawdust sample was used without any pretreatment except for drying. The weight of the dried sawdust was reduced to about 75% after drying in a microwave oven. Monoterpenes, limonene, α-pinene, and β-pinene (3 µL) first were vaporized by heating during injection, and then 3 µL TMA solution was vaporized, followed by monitoring the TMA concentration over 3 hours.

2.3 GC-MS analysis

The GC-MS analyses were performed using a JEOL JMS-Q1050 GC instrument, equipped with an HP-5ms column (30 m × φ 0.25 mm, film thickness 0.25 µm). The column oven was held at 40°C for 3 min, then increased to 300°C at a rate of 5.0°C/min and then held at 300°C for 5 min. The components were identified by comparison of their mass spectra with a NIST11 standard reference database, and α-pinene, β-pinene, limonene, α -terpineol, bornyl acetate, and terpinyl acetate were also identified by comparison of retention times with those of authentic samples.

2.4 NMR analysis

NMR spectra were recorded on a Brucker AVANCE 400 spectrometer at 30°C. Chemical shifts for \(^1\)H and \(^13\)C spectra were reported relative to tetramethylsilane (TMS) at 0 ppm. Assignments were made using two-dimensional correlation spectroscopy (COSY), nuclear Overhauser enhancement spectroscopy (NOESY) and \(^1\)H-\(^13\)C hetero-scalar correlation spectroscopy (HETCOR). Concentration of monoterpenes was approximately 80 mM in acetone-\(d_6\). Changes in the chemical shifts in the \(^1\)H-NMR spectra of monoterpenes in the presence of TMA were determined by addition of 1, 5, and 10 µL TMA solution.

2.5 Computational Chemistry Analysis

Modeling of the molecular structures of the monoterpenes and TMA was performed using Monomer Modeler and COGNAC in JSOL OCTA and J-OCTA 4.0\(^{13}\). The calculations of electric charges of the models were performed using Firefly (PC GAMESS) ver.
8.2.0. The density functional theory (DFT) using B3LYP / 6-31G* was used for calculations as a condition.

3. Results and Discussion

A decrease in TMA concentration with time appeared to be a result of adsorption onto the wall of the apparatus (Fig. 2). A large error was detected at 30 min, but the error decreased after 1 hour, indicating that the TMA vapor equilibrated after 1 hour.

The deodorizing effect of Japanese cypress sawdust on TMA is shown in Fig. 3. The TMA concentration after 3 hours injection of TMA vapor decreased with increasing sawdust amount and decreased by 75% when using 3 g sawdust, which demonstrate the deodorizing effect on TMA by the sawdust. Thus, the waste sawdust produced in a sawmill can be useful as a deodorant for fish odors.

The physical adsorption of TMA to micro pores on the sawdust surface, similar to activated carbon, was first proposed as the deodorizing mechanism. However, the concentration of TMA decreased only slightly compared with the control, even when using 3 g dried sawdust despite the increased surface area (Fig. 3). In this experiment, a compound in Japanese cypress that masked the odor was investigated as a deodorizing mechanism.

The GC-MS analysis was performed to identify components of the essential oil of Japanese cypress (Table 1). The volatile monoterpenes, α-pinene, β-pinene and limonene, were identified and used to demonstrate the deodorizing effect of TMA.

The TMA concentration was determined at 3 hours after injection of each monoterpenene (Fig. 4). For every

| Compound        | Molecular Weight | Retention Time [min] | Area [%] |
|-----------------|------------------|----------------------|----------|
| α-pinene        | 136              | 8.08                 | 16.33    |
| β-pinene        | 136              | 9.29                 | trace    |
| limonene        | 136              | 11.09                | 0.74     |
| α-terpinolene   | 136              | 13.01                | 0.35     |
| trans-borneol   | 154              | 15.19                | 0.4      |
| 4-terpineol     | 154              | 15.39                | 0.93     |
| α-terpineol     | 154              | 16.04                | 0.58     |
| bornyl acetate  | 196              | 18.44                | 1.15     |
| terpinyl acetate| 196              | 20.25                | 3.02     |
| α-coapene       | 204              | 21.06                | 1.1      |
| β-elemene       | 204              | 21.32                | 4.16     |
| isocaryophyllene| 204              | 22.13                | 0.48     |
| α-lumulene      | 204              | 23.04                | 0.67     |
| γ-murolene      | 204              | 23.39                | 6.23     |
| β-cubeene       | 204              | 23.44                | 1.52     |
| β-selinene      | 204              | 23.52                | 1.03     |
| α-amorphene     | 204              | 24.13                | 8.62     |
| γ-cadinene      | 204              | 24.33                | 13.48    |
| δ-cadinene      | 204              | 24.47                | 25.23    |
| α-cadinene      | 204              | 25.06                | 1.31     |
| T-murolol       | 222              | 27.30                | 4.16     |
| δ-cadinol       | 222              | 27.36                | 0.72     |
| α-cadinol       | 222              | 27.47                | 3.08     |

* listed the compounds shown the similarity value above 950 by NIST 11 standard reference database.
* identified with the retention time of authentic samples.
* sum of individual area was 95.3%.
monoterpene. TMA concentration decreased when compared to the control. β-pinene was the most effective, and reduced TMA concentration by 50%. Limonene and α- and β-pinenes could remove TMA, with β-pinene having the most effective effect.

For ammonia removal, N⋯H-hydrogen bonding between ammonia and limonene was indicated by the NMR spectra\(^9\). Both ammonia and TMA have an unshared electron pair and are electron donors. The influence of TMA on chemical shifts changes in the \(^1\)H-NMR spectra of monoterpenes were determined. The chemical shifts in the \(^1\)H-NMR spectra of limonene, α-pinene, and β-pinene (Fig. 5), and their changes in the presence of TMA, are shown in Table 2.

Upon addition of TMA, some double-bond protons or protons of methyl groups adjacent to double bond shifted slightly to lower magnetic field for every monoterpene (Table 2). In contrast, some methylene and methine protons showed a slight shift to high magnetic field for every monoterpene. This trend in chemical shift changes became clearer with increasing TMA concentration (Fig. 6). However, the \(\Delta \delta\) values were too small to form hydrogen bonds between TMA and the monoterpenes. Intermolecular NOE peaks between TMA and each monoterpene were not detected using NOESY (data not shown).

Therefore, the electrical charges of monoterpenes were determined by DFT calculations using OCTA and J-OCTA\(^{11-13}\). The electric charges of C and H in limonene are shown in Fig. 7. The values of H were all positive and larger than those of C because of the smaller electron negativity.

The values of H in limonene were compared with the chemical shift changes in the \(^1\)H-NMR spectra. Averaged values were used for CH\(_2\) (H4, 7, H5, 6, and H8, 11) and CH\(_3\) (H9 and H10) groups (Fig. 8). Values for the electric charge of double-bond protons (H1, 2) and its neighboring protons (H9 and 10) were larger than those of others. Fig. 6 (C) shows that the protons shifted to lower magnetic field were H1, 2, 9, and 10, which agreed well with the large electrical charge of the protons.

These large positively charged protons in limonene are attracted to the negative charge of TMA, and the target protons are unsheilded and so slightly shifted to low magnetic field. In contrast, the protons shifted to higher magnetic field (H3, 7, and 11) agreed with small electrically charged protons.

Similarly, the same electrical charge calculations of α-pinene and β-pinene showed that double-bond protons (H1) had a greater positive charge than that of other protons. The H1 was the proton showing the greatest shift toward low magnetic field for both compounds [Fig. 6 (A) and (B)].

Consequently, the positive charge of the monoterpine attracts the negative charge of TMA rapidly, followed by changes in electrical charge and/or small

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Fig. 5 Structures of monoterpenes and proton numbers.

Fig. 6 Chemical shift changes in the \(^1\)H-NMR spectra for α-pinene (A), β-pinene (B) and limonene (C) upon addition of TMA (1 µL: --- △ ..., 5 µL: --- × ..., 10 µL: --- ○ -- ). *H4 and H5 of limonene overlapped with TMA peaks.
conformation changes in the monoterpene caused by the interaction. Presumably, this is the deodorant mechanism of the monoterpene on TMA, and the corpus of weak interaction is an identifying factor of the masking effect.

TMA deodorizing experiment and analysis of the deodorizing mechanism of the compounds other than monoterpenes used in this experiment are being performed to further elucidate the deodorizing effect of Japanese cypress.

4. Conclusion

TMA concentration decreased with increasing amount of Japanese cypress sawdust; thus, the waste sawdust produced in a sawmill can be useful as a deodorant for fishy odors. The volatile monoterpenes α-pinene, β-pinene and limonene were identified in essential oils of Japanese cypress, and every monoterpene decreased the TMA concentration when compared to the control. The results of NMR studies and computational chemistry analyses suggest the following deodorizing mechanism: the positive charge of the monoterpene attracts the negative charge of TMA, resulting in changes in the electric charge and/or small conformational changes in the monoterpenes.

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Key words: deodorant mechanism, masking effect, trimethylamine, monoterpenes, sawdust of Japanese cypress
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ヒノキのおがくずとモノテルペン類のトリメチルアミンに対する消臭効果

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要旨：ヒノキ（Chamaecyparis obtusa）のおがくずとモノテルペン類であるリモネン，α-ピネンそしてβ-ピネンはトリメチルアミンに対し消臭効果を示した。トリメチルアミン濃度は，おがくずの増量に伴い減少することがわかった。製材所で生じるおがくずが魚臭の除去に有効であることを示した。ヒノキの精油成分をGC-MSで分析したところ，リモネン，α-ピネンそしてβ-ピネンなどの揮発性のモノテルペン類が検出された。また，それらはトリメチルアミンに対し消臭効果を有し，中でもβ-ピネンが最も効果的であった。モノテルペンの消臭メカニズムの解析をNMRと電荷計算の実験により行った。NMRの実験から，どのモノテルペンにおいてもトリメチルアミンと弱いながらも相互作用していることが示唆された。また，モノテルペン分子の中で，最も低磁場シフトしたプロトンは二重結合のプロトンであった。モノテルペンの電荷計算から，二重結合のプロトンは他のプロトンに比べより大きな正電荷を持つことがわかった。これらのことから，モノテルペンの正電荷がトリメチルアミンの負電荷を攻撃し，その結果モノテルペンの電荷の変化や小さな構造変化を引き起こしていることが考えられた。これが，モノテルペン類のトリメチルアミンに対する消臭メカニズムの一因である可能性が示された。

キーワード：消臭メカニズム，マスキング効果，トリメチルアミン，モノテルペン類，ヒノキのおがくず

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