**NOTE**

**Characteristic Odor of the Japanese Liverwort (Leptolejeunea elliptica)**

Kazutoshi Sakurai\(^1\)\(^*\), Kenichi Tomiyama\(^2\), Yoshihiro Yaguchi\(^2\), and Yoshinori Asakawa\(^3\)

\(^1\) Region Resources Division, Shizuoka Cancer Center Research Institute, 1007, Shimonagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, JAPAN
\(^2\) Corporate Research and Development Division, Takasago International Corporation, 1-4-11 Nishi-Yawata, Hiratsuka, Kanagawa 254-0073, JAPAN
\(^3\) Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, JAPAN

Abstract: The volatile components produced by *Leptolejeunea elliptica* (Lejeuneaceae), which is a liverwort grown on the leaves of tea (*Camellia sinensis*), were collected and analyzed using headspace solid-phase microextraction–gas chromatography/mass spectrometry (HS-SPME-GC/MS). 1-Ethyl-4-methoxybenzene (1), 1-ethyl-4-hydroxybenzene (2), and 1-acetoxy-4-ethylbenzene (3) were identified as the major components together with several other phenolic compounds, including 1,2-dimethoxy-4-ethylbenzene, and 4-ethylguaiacol in addition to sesquiterpene hydrocarbons, such as *α*-selinene, *β*-selinene, *β*-elemene, and *β*-caryophyllene. GC/Olfactometry showed the presence of linalool, acetic acid, isovaleric acid, *trans*-methyl cinnamate, and *trans*-4,5-epoxy-(2E)-decenal, as the volatile components produced by *L. elliptica*.

Key words: *Leptolejeunea elliptica*, 1-ethyl-4-hydroxybenzene derivatives, GC/Olfactometry, characteristic odor

1 Introduction

Liverworts are rich sources of lipophilic terpenoids and aromatic compounds, which show interesting biological properties, such as antimicrobial, antifungal, antiviral, muscle relaxation, and antioxidant activities\(^1\)\(^–\)\(^3\). Some liverworts produce pleasant mossy, sweet mushroom-like, or pungent odors and very unpleasant rancid or manure-like odors\(^1\)\(^–\)\(^3\). Sakurai et al. have reported that the thalloid liverwort, *Marchantia paleacea* subsp. *diptera*, produces (S)-3-perillaldehyde when stressed\(^6\). One of the most characteristic liverworts possessing a pleasant odor is *Leptolejeunea elliptica*, which is a miniature-sized stem-leafy liverwort that grows on the leaves of *Camellia sinensis* and *Illicium anisatum* found in the Honshu, Shikoku, and Kyushu regions of Japan. Nakayama et al.\(^5\) and Toyota et al.\(^6\) have reported that the Japanese *L. elliptica* produces 1-ethyl-4-hydroxybenzene (2) and its derivatives, as well as a few monoterpenoids. We reinvestigated the volatile components produced by *L. elliptica* using HS-SPME-GC/MS analysis to verify the contribution of these volatile compounds to the complex odor displayed by this liverwort.

2 Materials and Methods

2.1 Plant material

*L. elliptica* was collected from Aioi-cho, Naka-gun, Tokushima, Japan in November 2017 by Y. Asakawa. A voucher specimen was deposited in the Institute of Pharmacognosy, Tokushima Bunri University.

2.2 GC/MS

*L. elliptica* (0.10 g) was peeled from the surface of tea leaves using a pair of tweezers and stored in a 20-mL vial which was completely closed. HS gas was absorbed on a PDMS/DVB type fiber (Supelco) at 60°C for 20 min. The fiber was then introduced into the injector of a 7890A GC system (Agilent Technologies) equipped with a BC-WAX column (20 m × 0.18 mm i.d., 0.18 μm film thickness). The oven temperature program was set at 45°C with an initial holding time of 1 min and then increased to 230°C at a heating rate of 20°C/min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min and split ratio of 10:1. A 5975C inert XL EI/CI MSD (Agilent Technologies) was operated using electron ionization at an ionization energy of 70 eV in scan mode. The injector and the ion...
source temperatures were set at 250 and 200°C, respectively. The retention indices were calculated relative to the C₇-C₂₇ alkanes. The compounds were identified using the Mass Finder 2.3 program⁷, NIST library⁸, FFNSC 2 library⁹, and mass spectra reported in the literature and our own library database.

2.3 GC/Olfactometry

The volatiles of *L. elliptica* were introduced into the injector of a 7890A GC system (Agilent Technologies) equipped with a BC-WAX column (20 m × 0.18 mm, i.d., 0.18 μm film thickness). The GC conditions were similar to those described earlier (section 2.2). The detector was separate from the FID and olfactory systems. The retention times observed for each component were controlled to be the same as those obtained using HS-GC/MS described earlier (section 2.2).

2.4 Antimicrobial activity

The methanol extract (100 μg/mL) of *L. elliptica* was tested against a series of microorganisms, including *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*, following a method described in literature¹⁰.

### 3 Results and Discussion

The HS-SPME-GC/MS TIC chromatogram obtained for *L. elliptica* is shown in Fig. 1. 1-Ethyl-4-methoxybenzene (1, 52%), 1-ethyl-4-hydroxybenzene (2, 14%), and 1-acetoxy-4-ethylbenzene (3, 4%) were identified as the major compounds. Although *L. elliptica* emits a very complex odor, neither the ionones from the tea leaves nor linalool and

### Table 1 Volatile compounds of the Japanese *L. elliptica* by HS-SPME-GC/MS.

| Compound                                      | RI   | Peak Area (%) |
|-----------------------------------------------|------|---------------|
| n-Pentadecane                                 | 1500 | 0.04          |
| 1-Ethyl-4-methoxybenzene (1)                  | 1510 | 51.71         |
| α-Cedrene                                     | 1561 | 0.35          |
| α-Barbatene*                                  | 1574 | 3.48          |
| β-Elemene                                     | 1584 | 0.65          |
| β-Caryophyllene                               | 1589 | 1.99          |
| Thujopsene                                    | 1614 | 0.11          |
| β-Barbatene*                                  | 1647 | 2.31          |
| 4-Methoxystyrene (4)                          | 1656 | 0.47          |
| γ-Selinene                                    | 1677 | 0.45          |
| n-Heptadecane                                 | 1700 | 0.44          |
| β-Selinene                                    | 1707 | 3.42          |
| α-Selinene                                    | 1711 | 4.09          |
| Germacrene A*                                 | 1744 | 0.70          |
| 1-Acetoxy-4-ethylbenzene (3)                  | 1793 | 4.44          |
| cis-Calamenene                                | 1813 | 0.05          |
| 1,2-Dimethoxy-4-ethylbenzene                 | 1856 | 0.33          |
| 4-Vinylphenyl acetate                         | 1921 | 0.07          |
| Phenol                                        | 1982 | 0.01          |
| 4-Ethylguaiacol                               | 2006 | 0.34          |
| (6E)-Nerolidol                                | 2026 | 0.06          |
| trans-Methyl cinnamate                        | 2054 | 0.01          |
| Elemol                                        | 2059 | 0.03          |
| 1-Ethyl-4-hydroxybenzene (2)                  | 2148 | 13.66         |
| 4-Acetylacetophenone                          | 2330 | 0.02          |
| 4-Vinylphenol                                 | 2365 | 0.10          |

*) tentatively identified

---

Fig. 1 HS-SPME-GC/MS TIC chromatogram of the volatile components of the Japanese liverwort, *Leptolejeunea elliptica*. 768

*J. Oleo Sci.* **69**, (7) 767-770 (2020)
safrole from the *Illicium* species, which are the host plants of *L. elliptica*, were observed. In addition, *L. elliptica* contains 4-ethylguaicol, one of the sex pheromones of cockroaches, 1,2-dimethoxy-4-ethylbenzene, phenol, and several sesquiterpene hydrocarbons and alcohols, as shown in Table 1. In New Caledonia, there are many endemic liverworts and the volatile components produced by *Leptolejeunea epiphylla* and *Colura leratii* were investigated by Coulerie and Asakawa. We then compared the major volatile components observed in the Japanese *L. elliptica* and the New Caledonian *Lejeuneaceae* family. The Japanese *L. elliptica* produces compound (1), its derivative (2), and compound (3) as the major volatile compounds, while the New Caledonian *L. epiphylla* produced compound (2) as the predominant compound. Interestingly, compound (1), which is the major volatile produced from the Japanese *L. elliptica*, was not found in both of the New Caledonian *Lejeuneaceae* species studied (*L. epiphylla* and *C. leratii*). A further significant chemical difference observed between the three *Lejeuneaceae* species was the presence of a large amount of 4-methoxystyrene (4) in *C. leratii*. In fact, compound (4) is one of the major components produced by the Tahitian liverwort (*Cyathodium foetidissimum*), which has already been published by our group.

*L. elliptica* emits a very complex citrusy, minty, floral, and anis-like odor in the presence of water and the remaining scent exhibits a moldy odor. The odor of dried *L. elliptica* is similar to tea and different from that displayed by the fresh material. However, linalool, ionones, and compounds derived from anthranilic acid were not identified in both the dried and fresh *L. elliptica* samples. Furthermore, we used GC/Olfactometry to clarify the unique and complex odor of *L. elliptica*. As a result, several compounds such as linalool (floral odor), acetic acid, isovaleric acid, trans-methyl cinnamate (faint woody odor), and trans-4,5-epoxy-2E-decenal (metallic odor) were detected in the volatile components produced by *L. elliptica*. A description of the odors of each of these compounds are described in Table 2.

4 Conclusions

We analyzed the volatile compounds produced by the Japanese *L. elliptica* and identified compounds (1)–(3) as the major components. The strange characteristic scent of this liverwort was mainly due to these three components. However, several mono- and sesquiterpenoids including linalool, cedrene, barbatene, β-caryophyllene, β-elemene, selinenes, and nerolidol, as well as other volatile phenolic components exhibiting a moldy medicinal odor, such as vinyl phenol, vinyl guaiacol, ethyl guaiacol, and cresol, which were detected using GC/Olfactometry, also contribute to the unique scent of this liverwort.

### Table 2  Characteristic odorants of *L. elliptica* by HS-SPME-GC/Olfactometry.

| RI (BC-WAX) | Odorant            | Odor description     | Odor strength |
|-------------|--------------------|----------------------|--------------|
| 1026        | Unknown            | steamed rice-like    | ++           |
| 1324        | 2-Acetyl-1-pyrrole* | grocery             | ++           |
| 1431        | Acetic acid        | acid                 | ++           |
| 1531        | Linalool*          | green, floral        | ++           |
| 1651        | Isovaleric acid*   | sweaty               | ++           |
| 1977        | *trans*-4,5-Epoxy-(2E)-decanal | waxy, clay   | ++           |
| 2006        | 4-Ethylguaicol     | phenolic, medicinal  | ++           |
| 2054        | *trans*-Methyl cinnamate | medicinal | ++           |
| 2056        | 4-Cresol = p-Cresol | moldy                | ++           |
| 2064        | 3-Cresol*          | moldy, medicinal     | +++          |
| 2148        | 1-Ethyl-4-hydroxybenzene | moldy, medicinal | +++          |
| 2167        | 4-Vinylguaicol*    | moldy, medicinal     | +++          |
| 2194        | 5-Vinylguaicol*    | medicinal            | ++           |
| 2365        | 4-Vinylphenol      | moldy                | ++           |

*) tentatively identified. +++: very strong, ++: strong.
Acknowledgements

The authors thank to Dr. Takuya Ito (Osaka Ohtani University) for his help on the antimicrobial test of the crude methanol extract of *L. elliptica*.

References

1) Asakawa, Y. Chemical constituents of the Hepaticae. in *Progress in the Chemistry of Organic Natural Products*. Vol. 42, Springer, Vienna, pp. 1-285 (1982).
2) Asakawa, Y. Chemical constituents of the Bryophytes. in *Progress in the Chemistry of Organic Natural Products*. Vol. 65, Springer, Vienna, pp. 1-618 (1995).
3) Asakawa, Y.; Ludwiczuk, A.; Nagashima, F. Chemical constituents of Bryophytes: Bio- and Chemical Diversity, Biological Activity, and Chemosystematics. in *Progress in the Chemistry of Organic Natural Products*. Vol. 95, Springer, Vienna, pp. 1-796 (2013).
4) Sakurai, K.; Tomiyama, K.; Kawakami, Y.; Ochiai, N.; Yabe, S.; Nakagawa, T.; Asakawa, Y. Volatile emitted from the liverwort *Marchantia paleacea* subsp. diptera. *Nat. Prod. Commun.* 11, 263-264 (2016).
5) Nakayama, M.; Matsuo, A.; Kami, T.; Hayashi, S. Volatiles from *Leptolejeunea elliptica*. *Phytochemistry* 18, 328 (1979).
6) Toyota, M.; Koyama, H.; Asakawa, Y. Volatile components of the liverworts *Archilejeunea olivacea*, *Cheirolejeunea imbricata*, and *Leptolejeunea elliptica*. *Phytochemistry* 44, 1261-1264 (1997).
7) Konig, W.A.; Joulain, D.; Hoschmuth, D.H. 226-228 (2013). Available from: http://www.massfinder.com/
8) Web Book of Chemical Data from NIST. Available from http://www/cjemweb.com/
9) Joulain, D.; Konig, W.A. The Atlas of Spectral Data of Sesquiterpene Hydrocarbons, E.B.-Verlag, Hamburg (1998).
10) Sakurai, K.; Tomiyama, K.; Kawakami, Y.; Yaguchi, Y.; Asakawa, Y. Characteristic scent from the Tahitian liverwort, *Cyathodium foetidissimum*. *J. Oleo Sci.* 67, 1265-1269 (2018).
11) Sreng, I. Seducin, male sex pheromone of the cockroach *Nauphoeta cinerea*: Isolation, identification and bioassay. *J. Chem. Ecol.* 16, 2899-2912 (1990).
12) Coulerie, P.; Thouvenot, L.; Nour, M.; Asakawa, Y. Chemical originalities of New Caledonian Liverworts from *Lejeuneaceae* family. *Nat. Prod. Commun.* 10, 1501-1504 (2015).