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RAPID COMMUNICATION

Enantiomeric analysis of monoterpenes in Oba-kuromozi (*Lindera umbellata* var. membranacea)

Risa Inoue[1] · Koetsu Takahashi[1,2] · Yoshiaki Iiduka[1] · Daisuke Arai[1] · Tatsuya Ashitani[1,2]

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Introduction

Kuromozi (*Lindera umbellata* Thunb.) is an aromatic shrub that grows in Japanese forests, and it is used to prepare toothpicks and essential oils, the latter of which are important for the future of the forest products industry. Oba-kuromozi (*L. umbellata* var. membranacea) is a variation of the kuromozi found commonly in northeastern Japan (the Tohoku region) [1]. Recently, Japanese oak wilt, which is a disease mediated by the *Platypus quercivorus* beetle, has damaged oak forests in northeastern Japan [2], and it has been observed that deciduous shrubs, such as Oba-kuromozi, grow predominantly in the canopy gaps generated after Japanese oak wilt disease [3, 4]. Thus, Oba-kuromozi is promising as a useful forest resource in northeastern Japan.

Hayashi et al. [5] reported variations of the components of the essential oils obtained from *L. umbellata* Thunb., *L. umbellata* var. membranacea, *L. umbellata* var. lancea (Hime-kuromozi), *Lindera sericea* (Ke-kuromozi), and *L. sericea* var. glabrata (Usuge-kuromozi). The Oba-kuromozi essential oil consists mainly of monoterpenes, and it contains commercially important compounds, especially the fragrant compound linalool [6]. Some monoterpenes contain both (+) and (−) enantiomers in plant extracts, although the enantiomeric ratio differs in different plant species [7]. Linalool also contains both enantiomers in plant extract [6, 8]. The biological activities of linalool have been reported in racemate and each isomer, e.g., antileishmanial activity in *rac*-linalool [9], anesthetic activity in (S)-(+)-linalool [10], and acaricidal activity in (R)-(−)-linalool [11]. These enantiomers have different fragrance property [6, 8] and biological activity between (R)-(−)-linalool and (S)-(+)-linalool [12]. Therefore, it is important to analyze enantiomeric ratios of terpenoid in extracts for utilization of plant components and elucidation of plant physiology. The enantiomeric ratios of monoterpene also differ between different plant tissues, including the bark, leaves, and heartwood, or between intact and non-intact plant parts [13, 14]. However, details of the enantiomeric variations of the components of Oba-kuromozi oil were lacking. Therefore, in this study, we conducted a preliminary enantiomeric analysis of the monoterpene components of Oba-kuromozi.

Materials and methods

Preparation of extracts

Plant samples were collected from the Yamagata Field Science Center (Faculty of Agriculture, Yamagata University, Japan) on 26 October 2016 in Tsuruoka, Japan. Branches with leaves were cut from three Oba-kuromozi plants. The leaves were cut into 5-mm fragments. Branches less than 5 mm in diameter were classified as twigs and cut to less than 5-mm lengths after removing the leaves. Bark was peeled from branches of approximately 5–10 mm in diameter and shredded into roughly 5-mm lengths by scissors. After peeling away the bark, the woody part of the plants was cut to lengths of less than 5 mm. Five grams of each sample was extracted three times with 10–20 ml of hexane. Then, the three hexane extracts were combined and adjusted to a constant volume of 50 or 100 ml

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✉ Tatsuya Ashitani
ashitani@tds1.tr.yamagata-u.ac.jp

[1] Faculty of Agriculture, Yamagata University, 1-23 Wakaba-machi, Tsuruoka, Yamagata 997-8555, Japan

[2] The United Graduate School of Agricultural Science, Iwate University, Morioka 020-8550, Japan

by adding hexane, and 1 µl of the extract solution was injected into a gas chromatograph to analyze its terpene components.

Authentic compounds

Standard compounds of (+)-α-pinene, (−)-α-pinene, (−)-β-pinene, (−)-α-phellandrene, (+)-3-carene, 1,8-cineole, γ-terpinene, terpinolene, piperitone, carvacrol, β-caryophyllene, and nerolidol were purchased from the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Camphene, (+)-limonene, (±)-linalool, (±)-camphor, (−)-terpinen-4-ol, α-terpineol, and geraniol were purchased from the Kanto Chemical Industry Co., Inc. (Tokyo, Japan). (−)-Linalool was purchased from Sigma–Aldrich (St. Louis, MO, USA).

Analysis of compounds

Gas chromatography–mass spectrometry (GC–MS) data were collected with a Shimadzu (Kyoto, Japan) QP-2010 Ultra gas chromatograph–mass spectrometer under the following conditions: an Rtx-5 capillary column [30 m × 0.32 mm inner diameter; 0.25-µm film thickness; Restek (Bellefonte, PA, USA)], a column temperature ranging from 50 °C (1 min) to 320 °C (4 min) at 5 °C/min, an injection temperature of 230 °C, an interface temperature of 320 °C, and an acquisition mass range of 50–450 atomic mass units (EI, 70 eV) using helium as the carrier gas. Each peak in the chromatogram was identified by comparing the GC–MS data with authentic compounds or a reference [15].

An enantiomeric analysis was performed using a gas chromatograph–mass spectrometer attached to a CycloSil-B capillary column (30 m × 0.32 mm inner diameter, 0.25-µm film thickness; Agilent, Santa Clara, CA, USA) for a chiral compound analysis, a column temperature ranging from 60 to 200 °C (0 min) at 4 °C/min and from 200 to 240 °C (2 min) at 10 °C /min, an injection temperature of 230 °C, and an interface temperature of 240 °C. A GC-flame ionization detector analysis was performed with a Shimadzu GC-2014 gas chromatograph using the same capillary columns and temperature conditions as the GC-MS analysis and a detection temperature of 240 °C. The peak of each enantiomer was determined according to a reference [16]. The enantiomeric ratio was calculated by determining the percentage of one enantiomer peak area relative to the sum of both enantiomer peaks in a GC-flame ionization detector chromatogram.

Results and discussion

Variations of the component of different plant tissues

Figure 1 shows the total ion chromatograms of the mono- and sesquiterpenes obtained by the GC–MS analysis of sample no. 1. Mono- and sesquiterpenes in the woody part did not exist or were of very few amounts, because the extract of the woody part of the plant did not yield clear peaks in the chromatogram. The chromatograms of the other two plants yielded very similar results. The 1,8-cineol and linalool contents were higher than those of the other terpenes in the leaf extracts. In the twig and bark samples, linalool was detected clearly as a strong peak among the terpenes. While a chemical analysis of the essential oil of Oba-kuromozi has been reported, most studies focused on the essential oil of the leaves only or a mixture of leaves and branches. Furuhata et al. [17] reported differences in the components of kuromozi essential oil between the leaves and branches using an early GC instrument. However, until now, there were no studies of the components of the extracts of the leaves, barks, and twigs in Oba-kuromozi. Our results demonstrated that there are differences in the composition of the extracts of each tissue of the Oba-kuromozi plants. Therefore, preparing extracts from different plant tissues individually provide greater insights into the composition of the Oba-kuromozi oil.

Enantiomeric ratios of monoterpenes

The enantiomers of the terpene components were analyzed by a chiral GC column. α-Pinene, linalool, terpinene-4-ol, and α-terpineol contained both (+) and (−) enantiomers. There were no significant differences in the enantiomeric ratios between the three plants. The enantiomeric ratios of terpinene-4-ol and α-terpineol, which were detected only in the leaf extract, were approximately 20:80 ((+):(−)) and 10:90, respectively. (R)-(−)-terpinen-4-ol and (S)-(−)-α-terpineol were more abundant than their corresponding isomers. The enantiomeric ratio of α-pinene was approximately 90:10 ((+):(−)), and there were no significant differences in the enantiomeric ratio between the leaves, bark, and twigs. For the terpene compounds, we found clear differences in the enantiomeric ratio of linalool between the different plant tissues. Figure 2 shows the peaks of the linalool enantiomers in the gas chromatograms of each sample. The calculated enantiomeric ratios of linalool are shown Table 1. Leaves contained more (R)-(−)-linalool than (S)-(+)-linalool, and in contrast, the bark and twigs

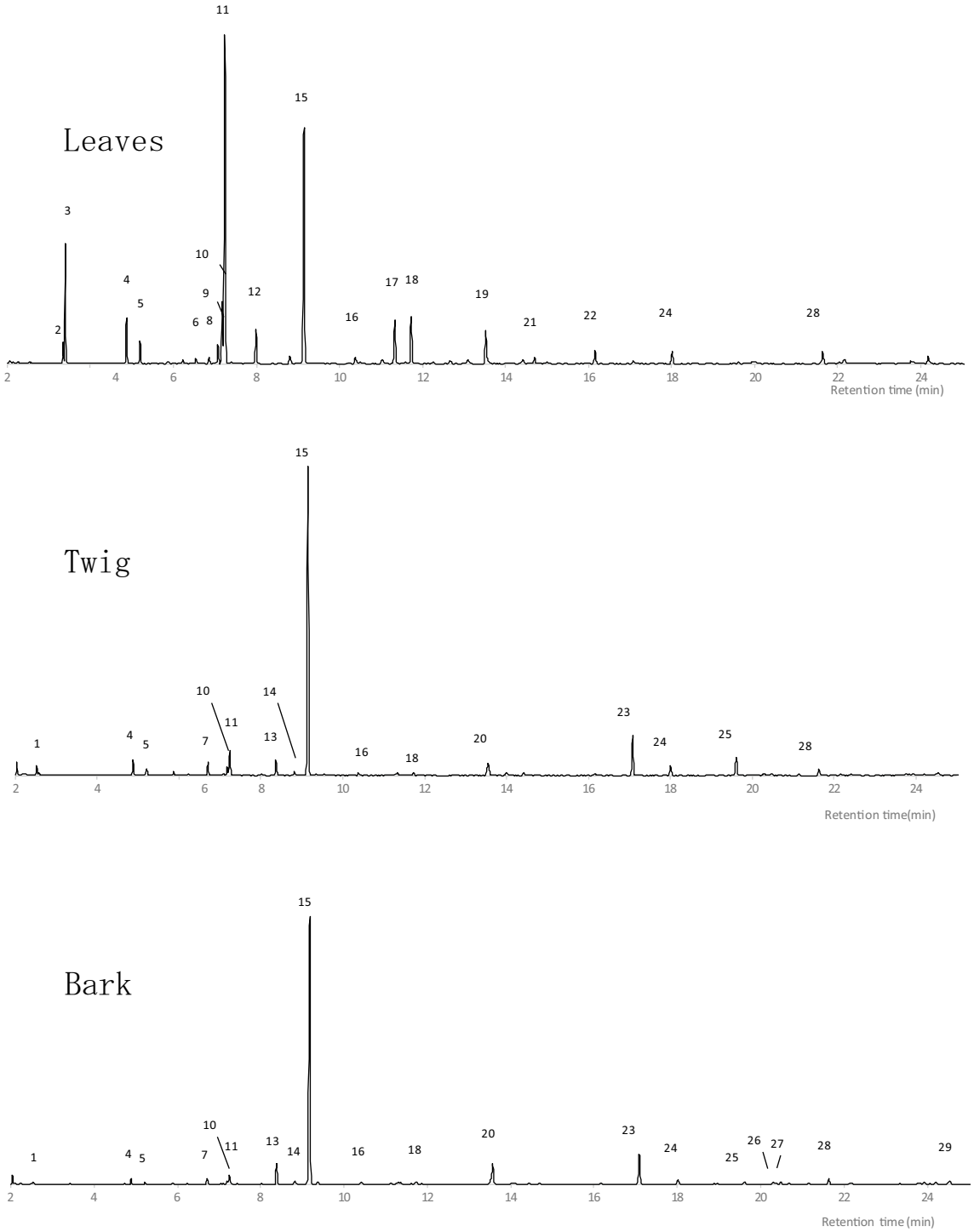

Fig. 1 Representative chromatogram of Oba-kuromozi hexane extracts obtained by a GC–MS analysis using an Rtx-5MS column. Note: The chromatograms show a magnification of the detected range of the terpenoid components. Peak identification: 1, 3-hexanol; 2, 2-hexenal; 3, 3-hexen-1-ol; 4, α-pinene; 5, camphene; 6, α-phellandrene; 7, 3-carene; 8, 2-carene; 9, *p*-cymene; 10, limonene; 11, 1,8-cineole; 12, γ-terpinene; 13, *cis*-furan linalool oxide; 14, *trans*-furan linalool oxide; 15, linalool; 16, camphor; 17, terpinen-4-ol; 18, α-terpineol; 19, piperitone; 20, geraniol; 21, carvacrol; 22, α-terpineol acetate; 23, geranyl acetate; 24, caryophyllene; 25, germacrene D; 26, β-bisabolene; 27, α-bergamotene; 28, nerolidol; and 29, α-bisabolol

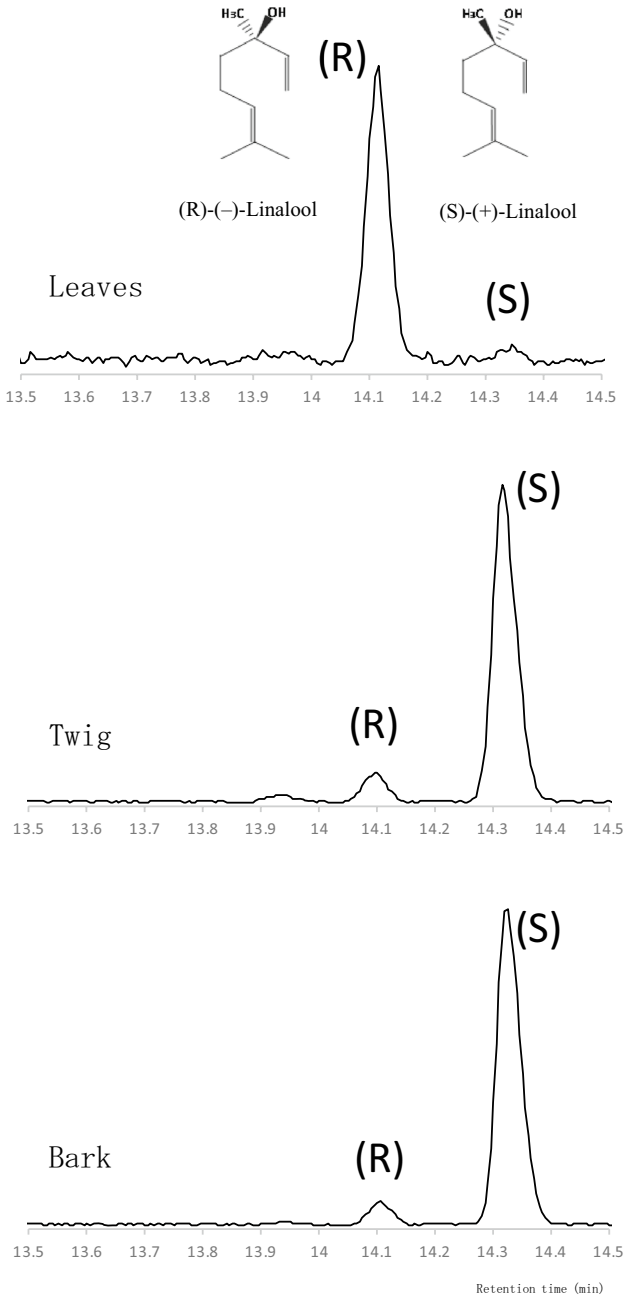

Fig. 2 Results of the enantiomeric analysis of linalool by GC–MS using a CycloSil-B column

contained more (S)-(+)-linalool than (R)-(−)-linalool. The biological activities such as fragrance property [6, 8] and microbial activity [12] differ between (R)-(−)-linalool and (S)-(+)-linalool. Although linalool is used as a fragrance, currently, the commercial product is racemic or (R)-(−)-excess linalool. Chemical and biological studies for (S)-(+)-linalool are comparatively difficult than (R)-(−)-linalool. The (S)-(+)-linalool of the bark and twigs of Oba-kuromozi will become important to efficiently use the extract components.

Table 1 Enantiomeric ratios of the linalool contained in each extract

Individual plants	Tissues	Enantiomeric ratio (%)	
		(S)-(+)-linalool	(R)-(−)-linalool
Sample no. 1	Leaves	4.6	95.4
	Twig	96.4	3.6
	Bark	96.6	3.4
Sample no. 2	Leaves	5.4	94.6
	Twig	92.3	7.7
	Bark	93.5	6.5
Sample no. 3	Leaves	22	78
	Twig	97.9	2.1
	Bark	97.7	2.3

Conclusions

We clarified the terpenoid composition of the different tissues of Oba-kuromozi and showed that there was a difference in the composition of the extracts of these tissues. The leaf extract had much greater amounts of 1,8-cineol and linalool among the monoterpenes. The bark and twig extracts had very similar compositions, and linalool was a main component in these extracts. For the terpene compounds, clear differences in the enantiomeric ratio of linalool were found between the different tissues. Leaves contained more (R)-(−)-linalool than (S)-(+)-linalool, and in contrast, the bark and twigs contained more (S)-(+)-linalool than (R)-(−)-linalool. Based on the different compositions of the extracts of the different tissues, in the future, it will be possible to efficiently collect specific compounds. The bioactivity of linalool has attracted great attention, but very few studies have examined its optical isomers. In the future, we will study the seasonal variations and bioactivities of the components of the different tissues, as well as other *Lindera* spp.

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