Volatile diterpene emission from dominant conifers in Japan

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Abstract

Reactive volatile organic compounds (VOCs) are known to affect atmospheric chemistry. Biogenic VOCs (BVOCs) have a significant impact on regional air quality due to their large emission rates and high reactivities. Diterpenes (most particularly, Kaur-16-ene) were detected in all of the 205 enclosure air samples collected over multiple seasons at two different sites from *Cryptomeria japonica* and *Chamaecyparis obtusa* trees, the dominant coniferous trees in Japan,. The emission rate of Kaur-16-ene, was determined to be from 0.01 to 7.1 $\mu g\ dwg^{-1}\ h^{-1}$ (average: 0.61 $\mu g\ dwg^{-1}\ h^{-1}$) by branch enclosure measurements and solid phase - liquid extraction techniques. The emission rate was an order of magnitude higher than that of monoterpenes, which is known major BVOC emissions, collected from same branches. In addition, total emission of Kaur-16-ene at 30 ℃ was estimated to be comparable to that of total anthropogenic VOC emissions. To our knowledge, this is the first report of volatile diterpene emissions from trees.

1. Introduction

Reactive volatile organic compounds (VOCs) have several important impacts on atmospheric chemistry and physics (Guenther et al., 2006). For example, VOCs are a precursor to the production of tropospheric ozone, an effective greenhouse gas known to be toxic at sufficiently high concentrations (Mustafa, 1990). Reactive VOCs at high concentrations may also decrease atmospheric concentrations of the hydroxyl radical (OH), a strong oxidant and major sink of less reactive greenhouse gases, such as methane (Kulmala et al., 2004). VOCs may also form secondary organic aerosols, which may produce direct and indirect effects on the earth's radiation budget (Kavouras et al., 1998).

VOCs are known to have both anthropogenic and biogenic sources. Biogenic VOCs (BVOCs) are mainly emitted from terrestrial plants and are estimated to contribute approximately 90% of total VOC emission on a global scale (Guenther et al., 2006). BVOCs often consist of unsaturated hydrocarbons, such as isoprene (C_5H_8), monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$) and oxygenated species such as methanol, 2-methy-3-butene-2-ol (methylbutenol) and others. However, it is suggested that there are still unmeasured constituents in the atmosphere (Di Carlo et al., 2004).

Coupled measurements of OH radical reactivity and VOC concentrations revealed that there is still a significant quantity of unmeasured reactant (most probably BVOCs) which cannot be detected by present analytical techniques (Di Carlo et al., 2004; Sinha et al., 2010). Studies revealed that there is a significant deviation between the observationally based and the atmospheric model based aerosol budgets, and is most likely caused by the existence of unmeasured compounds (Hallquist et al., 2009;

Beerling et al., 2011). These findings suggest the existence of unknown source(s) of reactive VOCs. On the other hand, a study based on enclosure sampling showed good agreement between OH reactivity and VOC concentration measurements (Kim et al., 2011). This suggests that emissions of unknown compounds may depend on the tree

5   species which is a common feature of biogenic emissions.

Diterpenes, a group of unsaturated biogenic hydrocarbons containing 20 carbon and 32 hydrogen atoms, have previously been identified in leaf oils and resins but not thought to be emitted from the vegetation due to their low volatility (Nakatsuka et al., 1957; Appleton et al., 1968, 1970; von Rudloff, 1975; Yasue et al., 1987; Cool et al.,

10   1998; Kato, 2005; Keeling and Bohlmann, 2006; Lee et al., 2009).). Oxygenated diterpenes, which contains one or more oxygen atoms besides carbon and hydrogen atoms, are also known. Thermo-desorption is one of the techniques most commonly used for BVOC measurements; however, it is unsuitable for the detection of high molecular weight compounds, such as diterpenes, because less volatile compounds

15   cannot be efficiently desorbed from the collection tubes. Thus, these compounds have been overlooked in previous studies. Consequently heavier BVOC may have been neglected by atmospheric scientists. In this study, we focused on heavier BVOC (diterpenes) and applied the solid adsorption - liquid extraction analytical technique (Matsunaga et al., 2011). The technique can elute heavy compounds such as diterpenes

20   effectively from the adsorbent, enabling the determination of emission rates of the heavier BVOCs. Kaur-16-ene and other compounds likely to be diterpenes were detected in all of the emission samples collected from *C. japonica* and *C. obtusa* over multiple seasons at two different sites. These two endemic tree species are the most dominant trees in Japan accounting for approximately 60% of total leaf mass in

Japanese forests (Matsunaga, 2011). Here, we present the first observations of biogenic emissions of diterpenes in significant concentrations.

Although the analytical technique cannot distinguish stereo-isomers compounds produced in leaves (Railton et al., 1984; Perry and Weavers, 1985; Cool et al., 1998), the Kaur-16-ene detected in this study is most likely to be ent-Kaurene. The emission rate of Kaur-16-ene was generally more than an order of magnitude higher than that of total monoterpene emissions. Assuming a temperature dependence of Kaur-16-ene emission used in the G93 model (Guenther et al. 1993), the emission was estimated to be comparable to total monoterpene emission, a group of commonly emitted BVOCs, and also to total anthropogenic VOC emission in Japan in summer.

2. Experiment

2.1. Site description and field campaign

Seven field campaigns have been conducted at two sites, Tanashi Experimental Station of the Tokyo University Forests (35º44'21"N, 139º32'18"E) in Tokyo and Miyazaki Research Forest of Kyushu University (32º21'23"N, 131º6'2"E) in Shiiba, Miyazaki, Japan. Both of the experimental forests have a planted area of mature *C. japonica* and *C. obtusa* trees. The campaigns were conducted in January, August, and November of 2009 and in April of 2010 at Tanashi and in May, August, and November of 2010 at Shiiba. Each sampling period was 5 days.

2.2. Branch enclosure system

Leaves were sampled at branch ends about 5-15 nodes from the trunk and were located at the top of the canopy approximately 10-15 m above the ground level. Tree

ages of *C. japonica* at Tanashi and Shiiba were 54 and 56 years, respectively, and for *C. obtusa*, at Tanashi and Shiiba were 33 and 56 years, respectively.

The branches were enclosed with a 15 liter Teflon® bag (transparent to more than 95% of PAR). The enclosure samples were collected from the branch of *C. japonica* at

5   canopy top and 5 m (3 m for *C. obtusa*) below the top within the same tree. The bags were installed at least 24 hours prior to the first sample collection to avoid disturbance due to the installation of the bag. The air inside the bag was ventilated with the preconditioned air, which did not contain VOCs and oxidant at significant concentrations, at a flow rate of 4-5 liter min^{-1}, to avoid water condensation and

10   excessive temperature increase in the bag (see Figure 1 for an overview). Therefore, contamination and degradation of the target BVOCs was be avoided during the sampling. The bags were installed onto the branches on the first day of the campaign and kept on the branches until the sampling period ended 5 days later. The air was supplied from a commercial compressed air cylinder and cleaned by an activated

15   charcoal trap. The flow rate was controlled by a mass flow controller. Air entering the bag was supplied through a Teflon® tube ring roughly 1.5 mm in diameter with approximately 20 holes so that the inside air could be circulated and homogenized. Adsorption of the diterpene onto the surface inside of the enclosure has been examined by the analysis of inside air of the bag immediately after removal of the branch. The

20   amount of diterpene adsorbed onto the surface was found to be around 0.1% of the amount of target compound collected within one hour of sampling time.

The compressed air was taken from the atmosphere and, thus, the CO_2 concentrations are assumed to remain at average ambient level. The maximum relative

humidity in the bag has been calculated to be 70-75% in the daytime. Water

condensation was observed when the air flow rate was reduced to below 4 liter min^{-1}.

Air temperature inside of the bag was recorded every 2 minutes by a data logger

equipped with a Teflon® coated thermo sensor (RS-12, Especmic, Ohguchi, Niwa, Aichi,

Japan). Branches were harvested after the completion of each field campaign and dried

at 60 ℃ for 48 hours to determine dry weight of the leaves. For *C. japonica*, the dry

weight was determined for whole branches harvested because stems also have green

needle leaves. Therefore, the dry weight for *C. japonica* includes stem, bark and leaves.

In the end, samples were collected from different branches of same individuals with

during sampling periods in every season.

2.3. Sample collection

Diterpene samples were collected from the enclosure bag described in 2.2.

Monoterpenes and sesquiterpenes, which are C_{10} and C_{15} BVOCs, respectively, were

also collected from the same bag and analyzed to confirm any disturbance (i.e.,

extraordinarily high concentrations from damage to storage reservoirs of the BVOCs).

The diterpene samples were collected into a 6.35 mm (1/4 inches) o.d. glass tube

filled with approximately 60 mg of HayeSep Q (Hayes Separations, Inc., Bandera TX,

U.S.A.). Sample flow rate was controlled at 200 ml min^{-1} by a mass flow controller

connected to an electronic pump. The collection time ranged from 0.5 to 2 hours.

Diterpenes collected on the adsorbent were extracted with approximately 2 ml of hexane

(Special grade, Wako chemical, Chuo-Ku, Osaka, Japan) at the site. The extracts were

collected into 2 ml glass vials (Supelco, Bellefonte PA, U.S.A.) directly from the

sampling tube and stored in a freezer (approximately -15 ℃), at the site. The samples

were transported to the laboratory by a commercial refrigerated transport service, which kept the packages at -18 ℃ before further procedures were performed.

2.4. GC-MS analysis

A one micro-liter of internal standard solution (20-100 ng μl^{-1} of cyclopentadecane in hexane) was added into the extracts prior to a concentration procedure. Thehe extracts were evaporated to approximately 5-10 μl by a gentle argon flow at the laboratory. The internal standard solution was prepared for each field campaign. One μl of the concentrated extract containing the internal standard was injected into the split-splitless injector of the gas chromatograph (GC; Agilent 7890A GC, Agilent Technologies Inc., Santa Clara CA, U.S.A.) connected to a mass spectrometer (MS; Agilent 5975C MSD). Diterpenes were separated using a fused silica capillary column (HP-5ms, 30 m × 0.25 mm i.d., film thickness of 0.25 μm; Agilent Technologies Inc.) and helium carrier gas. The injector (300 ℃) was equipped with a deactivated quartz insert tube and operated in the pulsed splitless mode. GC oven temperatures were programmed to start at 60 ℃ and increase to 150 ℃ at 2.5 ℃ min^{-1}, then raised to 300 ℃ at 30 ℃ min^{-1} and held for 10 minutes. The identification of Kaur-16-ene was made comparing its GC retention time and mass spectra with those of the authentic standard (Figure 2 and Figure 3). The authentic standard was purchased from OlChemIm Ltd., Olomouc, Czech Republic. Only Kaur-16-ene and an unknown diterpene, likely to be Hibaene, were detected as the two most abundant peaks of diterpenes (Figure 2).

Kaur-16-ene was quantified using the MS in the selected ion monitoring mode (SIM). The amount of the Kaur-16-ene was determined by comparing response intensity

(peak area on a chromatogram for the selected ions) of the internal standard and the

target compounds. The response of the Kaur-16-ene was adjusted based on the ratio of

the response for internal standard to Kaur-16-ene (different SIM ions were selected for

Kaur-16-ene and the standard). The ratio was determined by analyzing authentic

5   standard for Kaur-16-ene and internal standard (cyclopentadecane) purchased from

Wako and Sigma-Aldrich (Co., St. Louis MO, U.S.A.). The amount of the standard was

gravimetrically determined. The precision of the whole analytical procedure was around

5%. Detection limit was approximately 0.1 ng g^{-1} h^{-1} (depending on the leaf mass

enclosed in the bag and the sampling time).

10   2.5. Calculation for the emission rate of Kaur-16-ene

The emission rate was determined based on the equation 1 below.

$$E = \frac{F_{air} \times (M - M_{blank})}{F_{samp} \times t_{samp} \times W_{leaf}} \quad (1)$$

where, E and M are the emission rate in μg g^{-1} h^{-1} and determined mass of

Kaur-16-ene by the GC-MS analysis in μg, respectively. F_{air}, M_{blank}, F_{samp}, t_{samp} and

15   W_{leaf} are flow rate of the purified air supplied to the bag in ml min^{-1}, mass of

Kaur-16-ene in the blank sample in μg adjusted for the actual sampling time, sample

flow rate, sampling time in hour and dry weight of the leaf in g, respectively. M_{blank} for

Kaur-16-ene was below the detection limit through all field campaigns.

3. Results and discussion

20   3.1. Basal emission rate of Kaur-16-ene

Only Kaur-16-ene was identified and quantified in the diterpenes using an

authentic standard. To estimate the regional emission rate, the basal emission rate of Kaur-16-ene was calculated. Basal emission rate is defined to be an emission rate of BVOC, at the standard condition of 30 ℃ (Guenther et al., 1993, Guenther et al., 2006). Therefore, it is regarded to be a constant in most BVOC emission models. Figure 4a and 4b present observed relationships between the inside air temperature of the enclosure bag and measured emission rate of Kaur-16-ene from *C. japonica* and *C. obtusa* observed at Tanashi and Shiiba. Plot for *C. obtusa* was only for data obtained at Shiiba due to insufficient number of samples at Tanashi. Assuming a temperature dependence of the emission of Kaur-16-ene, the emission rate can be estimated according to the G93 algorithm (equation 2).

$$E = E_s \exp\{\beta(T - T_s)\} \quad (2)$$

$$\ln E = \beta(T - T_s) + \ln E_s \quad (3)$$

where, E presents measured emission rate. T and T_s presents measured temperature and the standard temperature, respectively. An empirical coefficient β can be calculated as the slope of the linear regression for the plot and quantifies the intensity of temperature dependence (equation 3). Natural logarithm of the basal emission rate E_s is the intercept at y-axis (Guenther et al., 1993). The calculated E_s and β over the seven campaigns are presented in Table 1. As shown in table 1, the basal emission rate E_s of Kaur-16-ene was significantly higher than those of monoterpenes, a more common BVOC emission, measured from same branches. Sesquiterpenes were detected only from *C. japonica*. The averaged basal emission rate of total sesquiterpenes from *C. japonica* was 2.9 and 7.1 µg g^{-1} h^{-1} at Tanashi and Shiiba, respectively, while that of Kaur-16-ene was determined to be 2.8 and 8.7 µg g^{-1} h^{-1}. Therefore, emission of

Kaur-16-ene is significant compared to those of mono- and sesquiterpenes which are commonly known BVOC emissions. Although there may be another factor which controls the emission (e.g. light intensity), we concluded that temperature is the most effective controlling factor of the emission of Kaur-16-ene, based on comparisons of the

5   emission rate between branch at the canopy top and the light attenuated branch. The basal emissions of Kaur-16-ene from *C. japonica* were 2.9 and 2.0 μg g^{-1} h^{-1} at sun exposed and shaded branches, respectively. Therefore, the light intensity is likely not an important factor to control the emission of Kaur-16-ene from *C. japonica*.

The basal emission rate of Kaur-16-ene did not show significant seasonal variation.

10   As mentioned above, basal emission rate is a normalized emission rate and is regarded to be a constant in most emission models because it is a normalized emission rate at a set of standard conditions. However, recent studies report that the basal emission often has a seasonal variation (Geron and Arnts, 2010). Matsunaga et al. (2011) also reports that the basal emission rates of monoterpenes and sesquiterpenes emitted from *C.*

15   *japonica* change with season. However, the basal emission rate of Kaur-16-ene emitted from *C. japonica* and *C. obtusa* did not clearly change with seasons. This may suggest that diterpenes are produced for other purposes or are produced through different processes than mono- and sesquiterpenes.

3.2. Estimate on the Kaur-16-ene emission over Japan

20   The total emission rate of Kaur-16-ene over Japan at 30 ℃ and 5 ℃ was based on leaf mass calculated from trunk volumes for *C. japonica* and *C. obtusa*. The trunk volumes are recorded in a statistical table of timber resources by each prefectural administration in Japan (Matsunaga, 2011). As shown in Figure 5, the emission rate of

Kaur-16-ene was estimated to be 630 and 3.3 ton h^{-1} at 30 ℃ and 5 ℃, respectively, while the emission rate of total monoterpenes (molecular weight; MW: 136) in Japan were estimated to be 250 tons h^{-1} at 30 ℃ and 26 tons h^{-1} at 5 ℃ by the emission model MEGAN (Model of Emissions of Gases and Aerosols from Nature) (Guenther et al., 2006). A similar emission rate is estimated by the MEGAN model, employing observed basal emissions for Kaur-16-ene and the coefficient β (460 and 2.7 ton h^{-1} at 30 ℃ and 5 ℃, respectively). It appears that the emission rate of Kaur-16-ene estimated at 30 ℃ exceeds the total emission rate of anthropogenic VOCs in Japan (220 ton h^{-1}) (Kannari et al., 2008). Considering the result, Kaur-16-ene can be regarded as a new and important atmospheric constituent that has a remarkable influence on the air quality in Japan. Because the ambient averaged temperature in Japan can be within the range of 5 and 30 ℃ and because the anthropogenic VOC emission does not significantly vary with temperature, the emission of Kaur-16-ene over Japan can be assumed to be comparable to that of anthropogenic VOCs.

4. Summary

4.1 Diterpene emission

A significant emission of diterpenes, which has not been recognized to be emitted into the atmosphere, has been determined employing both enclosure and ambient air measurements. Their reactivity is expected to be high due to their unsaturated structure and high molecular weight of molecules which contain many hydrogen atoms. In addition, the aerosol formation yield by the oxidation of diterpenes is also expected to be high due to their high molecular weight and reactivity. Considering the comparable emission rate to other classes of BVOCs such as monoterpenes and sesquiterpenes,

which are known to be major BVOC constituents, impacts of the diterpene emissions can be important in atmospheric chemistry and physics.

4.2 Unknown diterpene and future study

Samples also contained other diterpene-like compounds, which have not been identified by the authentic standards. One abundant unknown compound in the samples seemed to be Hibaene which is a diterpene also known as a constituent of coniferous leaves (Yatagai et al., 1985; Nagahama and Tajima, 1996). The mass spectrum obtained in this study was similar to that in NIST library. The Hibaene-like compound was detected in the samples at similar intensities with Kaur-16-ene. Therefore, total diterpene emission can be larger than presented in this study. Also, to confirm the importance of diterpene emissions at larger scales, other conifers such as *Abies, Larix, Picea* and *Pinus* spp., which are common at the global scale, should be investigated for the diterpene emissions in the near future.

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Figure captions

Figure 1 An overview for the branch enclosure sampling system.

Figure 2 Typical gas chromatogram of samples collected from *C. japonica*.

Figure 3 Structure and typical sample mass spectrum of Kaur-16-ene, analysis of the
5   authentic standard, and NIST library.

Figure 4 A liner relationship between the temperature and natural logarithm for the
          measured emission rate of Kaur-16-ene collected from (a) *C. japonica* and (b)
          C. obtusa (only for Shiiba).

Figure 5 Estimated emission rate of Kaur-16-ene at 30 ℃ over Japan.