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Purification and Gas Chromatography–Combustion–Isotope Ratio Mass Spectrometry of Aroma Compounds from Green Tea Products and Comparison to Bulk Analysis

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Supporting Information

ABSTRACT: A method for carbon isotope ratio ($\delta^{13}C$) analysis was developed for compound-specific isotope analysis of tea volatiles, and the values were compared with the $\delta^{13}C$ value from bulk isotope analyses. The $\delta^{13}C$ value of 2-phenylethanol liberated via enzymatic hydrolysis of the 2-phenylethyl β-primeveroside standard was examined first. Isotope fractionations for 2-phenylethyl β-primeveroside from preparative high-performance liquid chromatography (HPLC) were also analyzed. The enzymatic treatment and the preparative HPLC process did not cause carbon isotope fractionations, substantiating the strategies available for $\delta^{13}C$ analysis of volatile compounds. On the basis of the gas chromatography–combustion–isotope ratio mass spectrometry data from 2-phenylethanol, it was possible to derive the conditions for enzyme treatment and preparative HPLC of the glycoconjugates of 2-phenylethanol, (*Z*)-3-hexenol, and benzyl alcohol isolated from green tea leaves. Larger variations in $\delta^{13}C$ were found for individual volatile compounds compared with bulk analytical data from the leaves, indicating the potential to utilize this strategy in assigning the geographical origin of green tea.

KEYWORDS: *gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS), compound-specific isotope analysis, carbon isotope ratio ($\delta^{13}C$), enzymatic isotope fractionation, isotope fractionation in preparative HPLC, Camellia sinensis var. sinensis cv. Yabukita*

INTRODUCTION

Plants are classified into three types based on photosynthesis: C_3 ("normal" photosynthesis), C_4 (found in plants growing in water-restricted areas), and CAM (water-restricted plants that can survive in a dormant phase). C_3 plants utilize ribulose 1,5-bisphosphate carboxylase/oxygenase, whereas C_4 plants use phosphoenolpyruvate carboxylase for carbon fixation.[1] The different mechanisms of CO_2 fixation produce characteristic $\delta^{13}C$ values in the photosynthetic products, and thus, C_3 and C_4 plants can be distinguished by their $\delta^{13}C$,[2] thereby allowing the determination of whether manufactured food and materials are made from C_3 or C_4 plants.[3] $\delta^{13}C$ exhibits a weak temperature dependence but a direct relationship with water availability.[4] Although $\delta^{13}C$ has been proposed as a potential methodology in assigning the geographical origin of plants, very few studies have reported conclusive data.

In many previous studies, the $\delta^{13}C$ values of plants were based on bulk isotope analysis (of the whole plant). More recently, the number of applications concerning compound-specific isotope analysis of carbon and nitrogen has increased.[5,6] The growth environment of the plant potentially affects the levels of individual endogenous compounds, which may result in huge differences between samples causing $\delta^{13}C$ variation, based on bulk isotope analyses. Biosynthetic enzymes deplete ^{13}C to some extent in various metabolites,[7] resulting in a low $\delta^{13}C$ irrespective of whether the sample is from a C_3 or C_4 plant, suggesting that the $\delta^{13}C$ would be highly depleted from the value expected on the basis of the CO_2 assimilated in the initial photosynthetic step. By contrast, a $\delta^{13}C$ based on compound-specific isotope analysis may be unaffected by substances other than the compound of interest, even if the composition of each endogenous compound varies.

Green tea is the most popular beverage in Japan, appreciated by consumers mostly for its characteristic taste, color, and flavor. *Camellia sinensis* var. *sinensis* cv. Yabukita is the most common Japanese cultivar, with Shizuoka as the main production area. There are several other large tea-producing countries in the world, including Sri Lanka, Kenya, India, and China. International trade has led to the globalization of food markets and to growing consumer concerns about food quality and safety. Tracing the geographical origin of food is an important issue in

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this global food market, and several studies have focused on methodologies for the determination of food origin and authenticity.[8]

Large amounts of glycoconjugated alcoholic aroma compounds accumulate in the leaves of *C. sinensis* var. Yabukita as precursors of the final aroma compounds found in the tea.[9−11] During the production process of black and oolong teas, these glycoconjugates are hydrolyzed to yield a high concentration of aroma-active compounds. However, this hydrolysis does not occur in the green tea manufacturing process, making it difficult to obtain substantial amounts of volatile compounds from crude green tea samples for the investigation of the $\delta^{13}C$. Thus, a new experimental strategy for analysis of the $\delta^{13}C$ of volatile compounds from green tea has been developed, which consists of isolation of the glycoconjugates of each volatile compound by preparative high-performance liquid chromatography (HPLC) followed by an enzymatic hydrolysis process. Because a critical carbon isotope fractionation effect has been reported for the separation of organic compounds by preparative HPLC[12] and even enzymatic hydrolysis of vanilline glycosides caused carbon isotope fractionation,[13] we carefully examined the influence of the different sample preparation steps on the $\delta^{13}C$ values (i.e., HPLC separation and enzymatic cleavage reaction) required prior to compound-specific isotope analysis using nonvolatile glycoconjugates. 2-Phenylethyl β-primeveroside served as a model compound. On the basis of the optimized preparative HPLC and enzymatic hydrolysis conditions, three aroma glycoconjugates were isolated from green tea products, and the aglycones of each glycoconjugate were liberated via enzymatic hydrolysis to yield three volatile compounds. The compound-specific $\delta^{13}C$ values of the volatile compounds were analyzed via gas chromatography−combustion−isotope ratio mass spectrometry (GC−C−IRMS). The data were compared with the $\delta^{13}C$ from conventional bulk (tea products) isotope analyses.

MATERIALS AND METHODS

Chemicals. Chloroform (99.0% pure), methanol (99.8% pure), and sodium hydroxide (96.0% pure) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Ultrapure water was produced by a Direct-Q water-purifying apparatus (Millipore, Billerica, MA). Sodium citrate and citric acid for sodium citrate buffer were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The normal-alkane mixture B3 types with alkane $\delta^{13}C$ values of −30.66 ± 0.03‰ (C-16), −31.11 ± 0.02‰ (C-18), −32.35 ± 0.03‰ (C-20), etc., were obtained from A. Schimmelmann (Bremen, Germany).

Carbon Isotope Fractionation by β-Primeverosidase on the Hydrolysis of β-Primeveroside Standard Compounds of 2-Phenylethanol, (*Z*)-3-Hexenol, and Benzyl Alcohol. 2-Phenylethyl β-primeveroside standard (100 μg) was chemically synthesized in our laboratory[14] and treated in the presence of 50 mg of primeverosidase (Amano enzyme) in 50 mM sodium citrate buffer (pH 6.0, 1.0 mL) for 3, 6, 9, 12, 15, and 18 h at 37 °C. The 2-phenylethanol was extracted twice with dichloromethane (200 μL) and subjected to GC−MS and GC−C−IRMS analyses using 1 μL per injection. β-Primeverosidase treatment was also applied to (*Z*)-3-hexenyl β-primeveroside and benzyl β-primeveroside for 18 h at 37 °C in a similar manner, and the percent recovery of each aglycone part was estimated via GC−MS analyses.

Carbon Isotope Fractionation during HPLC Separation Based on the Peak Fractionation of 2-Phenylethyl β-Primeveroside Standard. 2-Phenylethyl β-primeveroside standard (100 μg) was subjected to HPLC separation (Prominence, Shimadzu) under the following conditions to give three fractions, Fr. A [t_R (retention time) = 26.0−26.8 min], Fr. B (t_R = 26.8−27.1 min), and Fr. C (t_R = 27.1−28.0 min) (Figure 1 of the Supporting Information): CAPCELLPAK ODS 20 mm × 250 mm column (Shiseido), flow rate of 10 mL/min, mobile phase A consisting of H_2O (0.05% formic acid), mobile phase B consisting of acetonitrile, isocratic 10% (v/v) B/(A + B), column temperature of 40 °C. The amounts of 2-phenylethyl β-primeveroside were very similar (31−34 μg) in each fraction.

The influence of sample preparation in preparative HPLC on the $\delta^{13}C$ value was also evaluated via a standard-based purification test. 2-Phenylethyl β-primeveroside standard (100 μg) was subjected to HPLC under the following conditions: CAPCELLPAK ODS 20 mm × 250 mm column (Shiseido), flow rate of 10 mL/min, mobile phase A consisting of H_2O (0.05% formic acid), mobile phase B consisting of acetonitrile, gradient of 10 to 35% (v/v) B/(A + B) over 20 min and 35 to 95% (v/v) B/(A + B) over 10 min, column temperature of 40 °C, detection at 200−350 nm. The standard that eluted with a t_R of 15.7 min was collected from 15.5 ($t_R - t_R/65$) min to 15.9 ($t_R + t_R/65$) min to give an HPLC-processed fraction of the original standard (Table 1).

Table 1. Analysis of $\delta^{13}C$[a] during the Processing of 2-Phenylethyl β-Primeveroside by HPLC

sample	$\delta^{13}C_{V\text{-}PDB}$ (‰)
original standard	−28.66 ± 0.14
HPLC-processed fraction of the original standard	−28.61 ± 0.20
peak fractionate test Fr. A	−24.61 ± 0.12
peak fractionate test Fr. B	−27.57 ± 0.04
peak fractionate test Fr. C	−34.90 ± 0.07
peak fractionate test Fr. A + Fr. B + Fr. C	−28.95 ± 0.11

[a]Carbon isotope ratios are reported as $\delta^{13}C$ ± the standard deviation (n = 3) in per mill (‰).

Each fraction was concentrated to dryness *in vacuo*, and the residue was dissolved in 1.0 mL of water. An aliquot (10 μL of the aqueous solution) of each fraction and the combined fractions (A + B + C) were treated in the presence of 50 mg of primeverosidase in 50 mM sodium citrate buffer (pH 6.0, 1.0 mL) for 12 h at 37 °C. The 2-phenylethanols were extracted twice with dichloromethane (200 μL) and subjected to GC−C−IRMS analyses using 1 μL per injection. The $\delta^{13}C$ was calculated as defined below.

Plant Materials. Tea plants (*C. sinensis* var. *sinensis* cv. Yabukita) were collected at Saitama, Shizuoka, Kyoto, Tanegashima, and Tokunoshima in Japan and at Zhejiang and Jiangsu in China as the first flush tea (Figure 2 of the Supporting Information). Each sample was steamed, rolled, and dried after being harvested as crude green tea in 2010. All samples were stored at room temperature.

Sample Preparation and Bulk Isotope Analysis. Whole tea products were crushed to obtain a powder for bulk isotope analysis. For $\delta^{13}C$ measurements, 1.0 mg of powder was loaded into tablet-shaped tin capsules (LÜDI SWISS AG, 5.0 mm × 9.0 mm, 0.15 mL).

The $\delta^{13}C$ was defined as $\delta^{13}C_{sample} = [(R_{sample}/R_{standard})-1] \times 1000‰$, where R_{sample} was the $^{13}C/^{12}C$ ratio in the sample and $R_{standard}$ was the internal standard $^{13}C/^{12}C$ ratio (Vienna Pee Dee Belemnite, VPDB) ($R_{standard}$ = 0.0112372). The bulk tea leaf $\delta^{13}C$ values were determined by cavity ring down spectroscopy combined with a combustion module (CM-CRDS, G1121-I analyzer, Picarro Inc., Santa Clara, CA). Acetanilide ($\delta^{13}C$ = −33.62‰) and L-threonine ($\delta^{13}C$ = −9.45‰) were chosen as carbon isotope references.

Isolation of Primeverosides of Volatile Compounds and Treatment with β-Primeverosidase Prior to the Compound-Specific Isotope Analysis. The tea samples (10 g) were crushed into powder and extracted with 100 mL of water while being sonicated for 30 min. The water extract was partitioned twice with 50 mL of dichloromethane to remove caffeine and low-polarity compounds. The water layers were combined and evaporated to dryness. The residues were dissolved in 10 mL of 50% aqueous MeOH, and 2-phenylethyl β-primeveroside, (*Z*)-3-hexenyl β-primeveroside, and benzyl β-primeveroside were purified via preparative HPLC under the following conditions: CAPCELLPAK ODS 20 mm × 250 mm column (Shiseido), flow rate of 10 mL/min, mobile phase A consisting of H_2O (0.05% formic acid), mobile phase B consisting of acetonitrile, gradient of 10 to 35% (v/v) B/(A + B) over 20 min and 35 to 95% (v/v) B/(A + B) over 10 min, and column temperature of 40 °C.

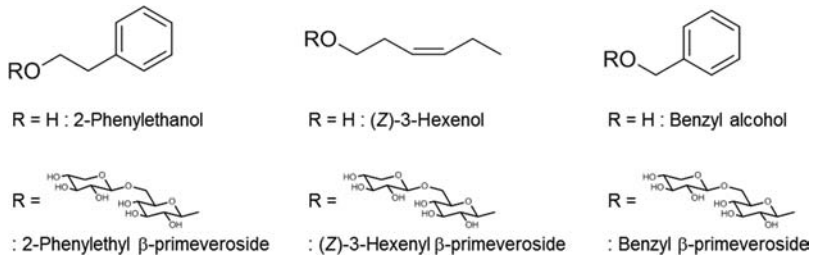

Figure 1. Chemical structures of 2-phenylethanol, (*Z*)-3-hexenol, benzyl alcohol, and their glycoconjugates (*β*-primeveroside).

The purification was guided by the chromatographic profiles of the stock specimens from our library: 2-phenylethyl *β*-primeveroside (t_R = 15.7 min), (*Z*)-3-hexenyl *β*-primeveroside (t_R = 16.2 min), and benzyl *β*-primeveroside (t_R = 13.1 min). The 2-phenylethyl *β*-primeveroside, (*Z*)-3-hexenyl *β*-primeveroside, and benzyl *β*-primeveroside fractions were collected from 15.5 to 15.9, 16.0 to 16.5, and 12.9 to 13.3 min, respectively (elution between $t_R - t_R/65$ min and $t_R + t_R/65$ min). Each glycoconjugate fraction was concentrated to dryness *in vacuo*, and the concentrate was dissolved in 1.0 mL of water. An aliquot (100 *μ*L of the aqueous solution) of each compound-rich fraction was treated in the presence of 50 mg of primeverosidase in 50 mM sodium citrate buffer (pH 6.0, 1.0 mL) for 12 h at 37 °C. The volatile compounds were extracted as mentioned above and subjected to GC−C−IRMS analyses using 0.1−1.0 *μ*g/*μ*L per injection. The δ^{13}C values were calculated as defined above.

Analytical Conditions. Quantitative analysis of 2-phenylethanol in the model experiments was performed using GC−MS (GCMS-QP5000, Shimadzu). The GC oven program was as follows: initial temperature of 60 °C held for 3 min and temperature then increased at a rate of 20 °C/min to 240 °C and then held at this final temperature for 3 min. Chromatography was performed on a SUPELCO WAX 10 column [SUPELCO, 30 m × 0.25 mm (inside diameter), 0.25 *μ*m film thickness]; the flow rate of the helium carrier gas was 1.6 mL/min. The injection port was set to 220 °C. The retention time of 2-phenylethanol was 634 s. The compound-specific δ^{13}C values of the aroma compounds were measured via GC−C−IRMS (Trace GC ultra gas chromatograph connected to a Delta V plus isotope ratio mass spectrometer, Thermo Fisher Scientific Inc., Bremen, Germany). The GC oven program was as follows: initial temperature of 35 °C held for 5 min and temperature then increased at a rate of 5 °C/min to 110 °C, increased at a rate of 10 °C/min to 240 °C, and held at this final temperature for 5 min.

Chromatography was performed on a SUPELCO WAX 10 column [SUPELCO, 30 m × 0.25 mm (inside diameter), 0.25 *μ*m film thickness]; the flow rate of the helium carrier gas was 1.0 mL/min. The combustion oven was set to 1030 °C. The helium backflush was set to five times per analysis. The retention times of 2-phenylethanol, (*Z*)-3-hexenol, and benzyl alcohol under these conditions were 1653, 1122, and 1628 s, respectively. All analyses were performed using Isodat version 3.0 (Thermo Fisher Scientific Inc.). Normal alkane mixtures of type B3 (obtained from A. Schimmelmann) were chosen as the carbon isotope reference.

RESULTS AND DISCUSSION

In GC−C−IRMS analysis, highly volatile organic substances are difficult to handle because of isotope fractionation associated with volatilization, oxidation, and cracking during sample preparation. Thus, nonvolatile glycoconjugates were obtained as precursors of aroma compounds, which are better suited for this purpose than the volatile compounds themselves. The conjugates are stable toward heat and light and are present in sufficient amounts for GC−C−IRMS analysis. The endogeneous levels of 2-phenylethyl, (*Z*)-3-hexenyl, and benzyl *β*-primeverosides in dried leaves (Figure 1) are 8.7, 6.1, and 34.4 mg/100 g, respectively.[15] Accurate isotope ratio measurements require the integration of the entire peak in GC analysis; therefore, it is important to completely separate genuine compounds from contaminants during the chromatographic run (Figure 2). In an effort to remove contaminants prior to GC−C−IRMS, the glycoconjugates were purified by HPLC, and enzymatic hydrolysis was then performed to extract exclusively the bound aroma compounds.

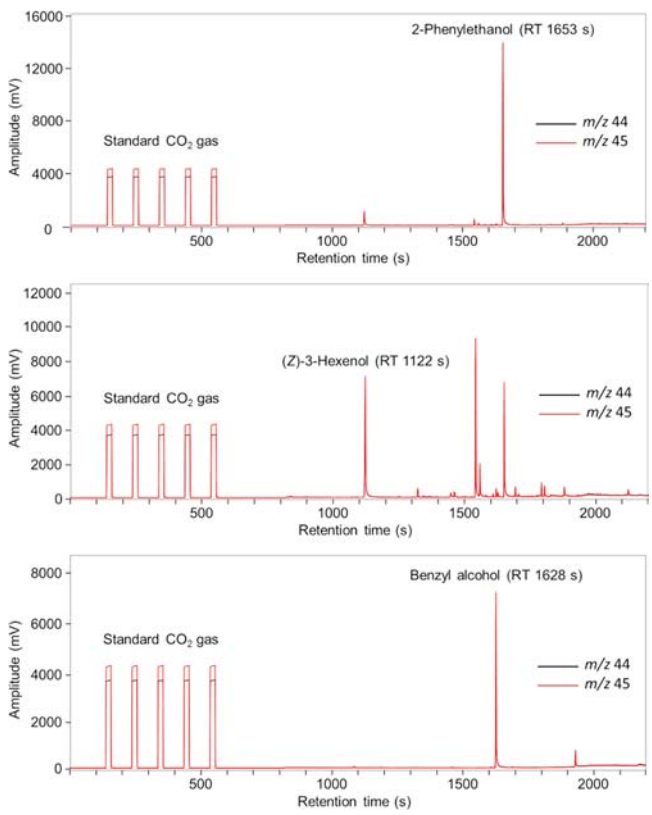

Figure 2. GC−C−IRMS chromatograms of aroma compounds produced via enzymatic hydrolysis of their glycoconjugates purified from Shizuoka crude tea sample: (top) 2-phenylethanol, (middle) (*Z*)-3-hexenol, and (bottom) benzyl alcohol.

This experimental strategy for analyzing the δ^{13}C values of volatile compounds requires an enzymatic hydrolysis process of the glycoconjugate of each volatile compound, because chemical hydrolysis under strongly acidic conditions sometimes alters the chemical structure of the aglycone portion of the molecule.[9] The conditions for enzymatic hydrolysis were determined by considering a possible fractionation of carbon isotopes: the relationship between enzyme reaction time and δ^{13}C was evaluated using a standard glycoconjugate, i.e., by enzymatic hydrolysis of 2-phenylethyl *β*-primeveroside with *β*-primeverosidase as the cleavage enzyme (Figure 3).[16] Within 18 h, the amount of enzymatically liberated 2-phenylethanol increased to

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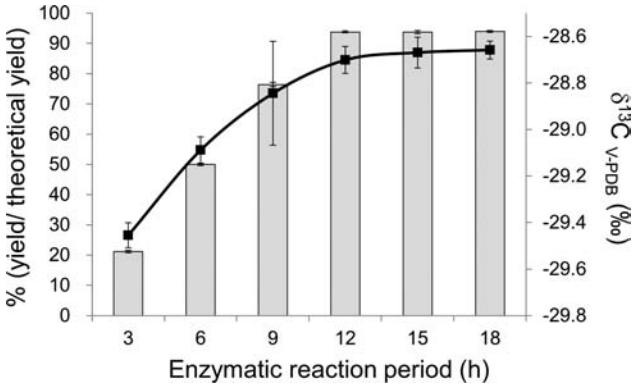

Figure 3. Change in the $\delta^{13}C$ of 2-phenylethyl β-primeveroside during an enzymatic hydrolysis reaction. Data are the means ± standard deviation (n = 3) for the $\delta^{13}C$ of 2-phenylethyl β-primeveroside (red line) and the amount of free 2-phenylethanol (reactant) from the glycoconjugate.

reach its maximum (93.8−94.0% yield) after 12 h, suggesting that most of the substrate was cleaved by primeverosidase by that time. On the basis of GC−MS analyses, the recovery of 2-phenylethanol was 94% after enzymatic hydrolysis followed by a CH_2Cl_2 extraction step. As shown in Figure 3, primeverosidase preferentially utilized ^{13}C-depleted 2-phenylethyl β-primeveroside as its substrate to yield 2-phenylethanol with $\delta^{13}C$ values of −29.45‰ after hydrolysis for 3 h and −28.70‰ after hydrolysis for 12 h. During this period, a sufficient amount of cleavage enzyme was present in the reaction mixture. After complete hydrolysis (12−18 h), the $\delta^{13}C$ value of 2-phenylethanol remained at a constant level of −28.68 ± 0.02‰). Primeverosidase hydrolyzed 2-phenylethyl, (Z)-3-hexenyl, and benzyl β-primeverosides to a similar extent, and there was no selectivity for these substrates.[17] Recoveries of (Z)-3-hexenol and benzyl alcohol were 95 and 96%, respectively. On the basis of these results, each primeveroside was subjected to hydrolysis for 12 h in the presence of a sufficient amount of primeverosidase to completely hydrolyze the tea glycoconjugates.

Chromatographic separation on a silica gel column has been reported to cause site-specific isotope fractionation of naturally occurring vanillin.[12] Preparative HPLC on zeolite shape-selective molecular sieves does not cause any carbon isotope fractionation.[18] Carbon isotope fractionation in a preparative HPLC process was then examined on an ODS column using a standard compound, 2-phenylethyl β-primeveroside, as a model experiment.

2-Phenylethyl β-primeveroside was passed through an ODS column followed by an enzymatic treatment and CH_2Cl_2 extraction. The 2-phenylethanol thus obtained was subjected to GC−C−IRMS analyses. No significant difference was detected compared to the 2-phenyethanol liberated from the original standard (Table 1, HPLC-processed fraction of the original standard).

The standard was then separated into three fractions (Fr. A, Fr. B, and Fr. C) via preparative HPLC developed with a more polar solvent, to ensure that the concentration of 2-phenylethyl β-primeveroside was almost identical in each of the three fractions. The 2-phenylethanol part liberated from fraction A had a less depleted ^{13}C than those from fractions B and C (Figure 1 of the Supporting Information and Table 1). This is known to be due to the isotopic chromatographic effect.[18]

The preparative HPLC procedures were therefore determined by collecting the fractions eluting between $t_R - 1/65t_R$ min and $t_R + 1/65t_R$ min for complete recovery. This preparative HPLC procedure excluded any isotopic chromatographic effects on the liberated aglycones. On the basis of the evaluation of carbon isotope fractionation during preparative HPLC as well as the process of enzymatic hydrolysis, we were able to optimize the method to obtain volatile compounds from green tea samples for analyzing accurate $\delta^{13}C$ values.

First, three aroma glycoconjugates, 2-phenyethyl, (Z)-3-hexenyl, and benzyl β-primeverosides from one tea product purchased in a local market in Shizuoka were purified via HPLC according to the method described above. After enzymatic hydrolysis with β-primeverosides for 12 h, the enzymatically liberated 2-phenylethanol, (Z)-3-hexenol, and benzyl alcohol were subjected to GC−C−IRMS. The $\delta^{13}C$ of the bulk (tea product) was −27.7‰, whereas the compound-specific analyses of each volatile compound showed more depleted $\delta^{13}C$ values, −28.1, −30.6, and −35.1‰, respectively (Table 2), suggesting that an enzymatic isotope fractionation occurred along the biosynthetic pathway of each volatile compound. In the seven geographic areas, the $\delta^{13}C$ of 2-phenylethanol, (Z)-3-hexenol, and benzyl alcohol ranged from −26.40 to −28.40‰ (Δ2.00‰), from −31.69 to −27.49‰ (Δ4.20‰), and from −36.55 to −28.80‰ (Δ7.75‰), respectively. Benzyl alcohol showed the largest differences, while 2-phenylethanol showed the smallest difference between the bulk material. Each compound had a characteristic $\delta^{13}C$ value range, as has been observed in essential oils.[19,20] As the three volatile compounds studied here were synthesized by different enzymes, characteristic $\delta^{13}C$ values were established through the unique isotope fractionation factor for each enzyme. Patterns across the range of values measured for the single compound and bulk material from various geographic area were similar (Table 2), and the ratios of the $\delta^{13}C$ from analysis of the bulk to the compound-specific isotope analysis are almost identical for each volatile compound in each geographic area (Figure 3 and Figure 4 of the Supporting

Table 2. $\delta^{13}C_{V\text{-}PDB}$ Values[a] of Individual Volatile Compounds and Bulk Plants from Several Growing Areas

	$\delta^{13}C_{V\text{-}PDB}$ (‰)			
crude tea production area	bulk	2-phenylethanol	(Z)-3-hexenol	benzyl alcohol
Saitama	−26.87 ± 0.19	−27.25 ± 0.32	−28.83 ± 0.39	−34.60 ± 0.12
Shizuoka	−27.73 ± 0.15	−28.07 ± 0.07	−30.60 ± 0.04	−35.07 ± 0.13
Kyoto	−28.28 ± 0.24	−28.41 ± 0.17	−31.02 ± 0.08	−36.55 ± 0.21
Tanegashima	−27.14 ± 0.13	−27.80 ± 0.19	−30.83 ± 0.06	−34.46 ± 0.19
Tokunoshima	−25.82 ± 0.15	−26.40 ± 0.20	−27.49 ± 0.20	−28.80 ± 0.01
Jianshu	−26.90 ± 0.17	−26.55 ± 0.05	−29.77 ± 0.20	−34.10 ± 0.02
Zhejiang	−26.84 ± 0.38	−27.58 ± 0.09	−31.69 ± 0.08	−35.28 ± 0.05

[a]Carbon isotope ratios are reported as $\delta^{13}C$ ± the standard deviation (n = 3) in per mil (‰).

Information). These results indicated that there is a common factor affecting the $\delta^{13}C$ values between the bulk (all of the compounds in the plant) and the volatile compound. The growth conditions (CO_2 concentration,[21] humidity, temperature, etc.) among seven different geographic areas may also influence the enzymatic activity involved in the biosynthesis of each compound, resulting in the different carbon isotope fractionation, and/or compositional change in each plant material.

This new method allows accurate compound-specific isotope analysis of many kinds of compounds without isotope fractionation of the desired compound, which is the basis for the development of methods for plant-based authenticity control. However, a larger database with at least three tea samples from each geographic area is necessary before this methodology can be applied to verification of tea authenticity based on $\delta^{13}C$ values.

■ ASSOCIATED CONTENT

Supporting Information

HPLC profile of 2-phenylethyl β-primeveroside and fractionation (Figure 1), map of the seven green tea production areas (Figure 2), values of ($\delta^{13}C$ of bulk)/($\delta^{13}C$ of volatile compound) in several areas (Figure 3), results from bulk analysis and compound-specific isotope analysis of 2-phenylethanol, (*Z*)-3-hexenol, and benzyl alcohol (Figure 4). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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