Quantitative Determination of β-Damascenone in Foods Using a Stable Isotope Dilution Assay

Alina Sen, Gudrun Laskawy, Peter Schieberle, and Werner Grosch*

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, 8046 Garching, West Germany

A stable isotope dilution assay has been developed for the quantitative determination of β-damascenone in foods. Application of the method to roasted coffee (powder and brew), black tea, honey, and beer has indicated that the odorant can be determined with a high degree of sensitivity and accuracy.

The odorant 1-(2,6,6-trimethyl-1,3-cyclohexadienyl)-2-buten-1-one (β-damascenone), isolated for the first time from Bulgarian rose essential oil (Demole et al., 1970), has been detected in various foods and stimulants (Maarse and Visscher, 1988). Apples (Nursten et al., 1972), black tea (Renold et al., 1974), wine (Schreier and Drawert, 1974), German draft beer, Pilsener type (Tressl et al., 1978), tomato (Buttery et al., 1988), honey (Blank et al., 1989), and coffee (Spadone et al., 1990) are some examples.

β-Damascenone smells sweet and honey-like (Kovats, 1987) with an odor threshold in water of 2 ng/kg (Buttery et al., 1988). This suggested that concentrations of β-damascenone down to the parts per thousand range might contribute significantly to the flavor of a food or stimulant.

A first approach to quantify β-damascenone in food was performed by Acree et al. (1981), who determined this compound in several cultivars of three species of grapes by mass chromatography. As the authors added the internal standard (1-chloronaphthalene) to a fraction obtained from a Florisil column, the loss of β-damascenone during extraction and chromatography was not taken into consideration.

As earlier shown (Schieberle and Grosch, 1987), a stable isotope dilution assay is an accurate method for the quantification of low concentrations of odorants. The aim of the following investigation was to develop such an assay for β-damascenone and to apply it for the analysis of roasted coffee, black tea, beer, and honey samples.

EXPERIMENTAL PROCEDURES

Materials. Roasted Arabica and Robusta coffee samples were obtained from a manufacturer. German lager beer, black tea, and honey samples were purchased from a local market. Boiled water (1.35 L) was poured on ground roasted coffee (72 g), and the suspension obtained was filtered through a paper filter, yielding 1 kg of a coffee brew.

Chemicals. β-Ionone (99%) was from C. Roth, GmbH, Karlsruhe, West Germany, and [^{2}H]acetone (99+ atom % ^{2}H) from Aldrich, Steinheim, West Germany. Silica gel 60 (Merck, Darmstadt, West Germany) was treated with HCl and deactivated with 4.8% (w/w) water according to the method of Esterbauer (1968).

Synthesis. Unlabeled β-damascenone was prepared by first constructing the trimethylcyclohexadiene ring system, followed by elaboration of the side chain, as suggested by Büchi and Wüest (1971). An intramolecular Wittig addition of allylidenetriphenylphosphorane to ethyl α-isopropylideneacetoacetate produced ethyl α-safranate. Exposure to acidic media led to a mixture of isomers containing 60% of the desired β-diene system, and condensation of the ethyl β-safranate with allyllithium afforded the title compound in nearly quantitative yield.

[^{2}H]-β-Damascenone was prepared by following the approach described above but required the synthesis of [^{2}H]ethyl α-isopropylideneacetoacetate. As specified by Kazi et al. (1964), the labeled keto ester was obtained in good yield by condensation of deuteriated acetone with ethyl acetoacetate in hot acetic anhydride containing zinc chloride. The compound was purified by distillation under a water jet vacuum.

Concentrations of Labeled and Unlabeled β-Damascenone. After the synthesis, the concentrations of labeled and unlabeled β-damascenone were determined gas chromatographically with β-ionone as the internal standard and using a SE-54 capillary (Blank et al., 1989).

Analysis. Extraction of the honey, roasted coffee, and tea samples required the following procedures: Honey (600 g) was diluted with 1.2 L of 0.2 mol/L sodium borate buffer (pH 9.0), which was saturated with sodium chloride. After addition of 5 μg of deuteriated β-damascenone, the pH of the honey solution was readjusted to 9.0 with aqueous 30% (w/v) KOH. The solution was stirred for 20 min and then extracted with dichloromethane (750 mL) by using a shaking machine (Blank et al., 1989). The roasted coffee (50 g) and the tea (400 g) samples were soaked overnight in diethyl ether and then extracted with this solvent for 7 h in a Soxhlet apparatus. The extracts obtained were spiked with 5–25 μg of deuteriated β-damascenone.

For extraction of the coffee brew (900 mL) and the beer (500 mL), after addition of 5 μg of [^{2}H]-β-damascenone, the sample was stirred (20 min) and then twice extracted with dichloromethane (250 mL portions).

Isolation of the Volatiles. After drying over sodium sulfate, the extracts were concentrated to about 120 mL by distilling off the solvent on a Vigreux column (50 × 1 cm). The sample was then poured into the distillation flask of the apparatus shown in Figure 1 and frozen for 30 min in liquid nitrogen. After sublimation of the volatiles and of the solvent in vacuo (0.02 Pa) for 3 h, the temperature of the water bath was increased to 50 °C, and the sublimation was continued for a further 2 h more. The condensates of the second and third cooling traps (6 and 7 in Figure 1) were combined and finally concentrated by microdistillation to about 200 μL for column chromatography.

Column Chromatography. The volatiles were fractionated at 10–12 °C on a water-cooled column (20 × 1 cm) packed with a slurry of silica gel 60 in pentane–diethyl ether (95:5 v/v). Stepwise elution was then performed with 25 mL of 95:5 pentane–diethyl ether (v/v;

* To whom correspondence should be addressed.

0021-8561/91/1439-0757$02.50/0

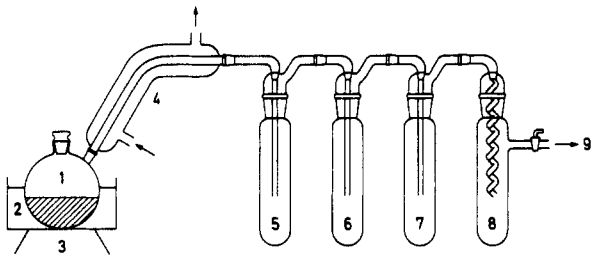

Figure 1. Apparatus for the isolation of the volatiles. (1) Two-necked distillation flask (volume: 1 L); (2) water bath; (3) magnetic stirrer; (4) tube with a water jacket which was held by a thermostat at the temperature of the water bath; (5–8) traps cooled with ice–water (5), carbon dioxide–methanol (6), and liquid nitrogen (7 and 8); (9) pump system which includes a two-stage rotary vane vacuum pump and an oil diffusion pump (Leybold-Heraeus, Koeln, West Germany).

fraction A) and 100 mL of 90:10 pentane–diethyl ether (v/v; fraction B). Fraction B was concentrated as reported above.

HPLC. Fraction B was separated by HPLC with the silica gel column (50 × 0.46 cm) and the apparatus described by Schieberle and Grosch (1988). Elution (flow rate: 2 mL/min) was performed with pentane–diethyl ether (97:3 v/v). The effluent in the range of 20–26 mL was collected. To obtain enough material, the effluents of several runs were combined, concentrated by microdistillation to 100 μL, and then analyzed by mass chromatography. After each run, 60 mL of pentane–diethyl ether (80:20 v/v) followed by 40 mL of pentane–diethyl ether (97:3 v/v) was pumped through the HPLC apparatus to purify and reequilibrate the HPLC column.

Quantification by Mass Chromatography. The samples (0.5 μL) were applied by the "on-column injection technique" at 35 °C on a 30 m × 0.32 mm fused silica capillary (DB-7, J&W, Carlo Erba, Hofheim, West Germany), which was coupled to the ion trap detector (ITD-800, Finnigan, Bremen, West Germany) running in the chemical ionization mode with methanol as reagent gas. The electron impact voltage was 70 eV, and the voltage applied to the electron multiplier was set at 1550 V. The temperature of the fused silica capillary was raised by 40 °C/min to 50 °C, held 2 min isothermally, and then raised by 6 °C/min to 230 °C. Ion abundances in the range of m/z 180–220 were monitored.

Comparison of the integrated abundance of the ion at m/z 191 of β-damascenone (I_{191}) to that of the sum of the abundances of the ions at m/z 196–198 of the [^{2}H]-β-damascenone ($I_{196-198}$) provided the data needed to carry out quantitative calibration of the method. The calibration factor R was calculated according to

$$R = \frac{\beta\text{-damascenone } (\mu g)\cdot I_{196-198}}{[^2\text{H}]\text{-}\beta\text{-damascenone } (\mu g)\cdot I_{191}}$$

The R values found in five trials with different ratios (1:10 to 10:1) of labeled and unlabeled β-damascenone (range: 4–45 μg) were averaged to get a working value of $R = 0.803 \pm 0.012$.

Capillary Gas Chromatography (HRGC)–Mass Spectrometry (MS) Analysis. HRGC–MS analyses of the synthesized β-damascenones were performed by using an MS 8230 (Finnigan) in tandem with an OV-1701 capillary (Schieberle and Grosch, 1988). Mass spectra in the electron impact mode (MS-EI) were generated at 70 eV and in the chemical ionization mode (MS-CI) at 115 eV with isobutane as reagent gas.

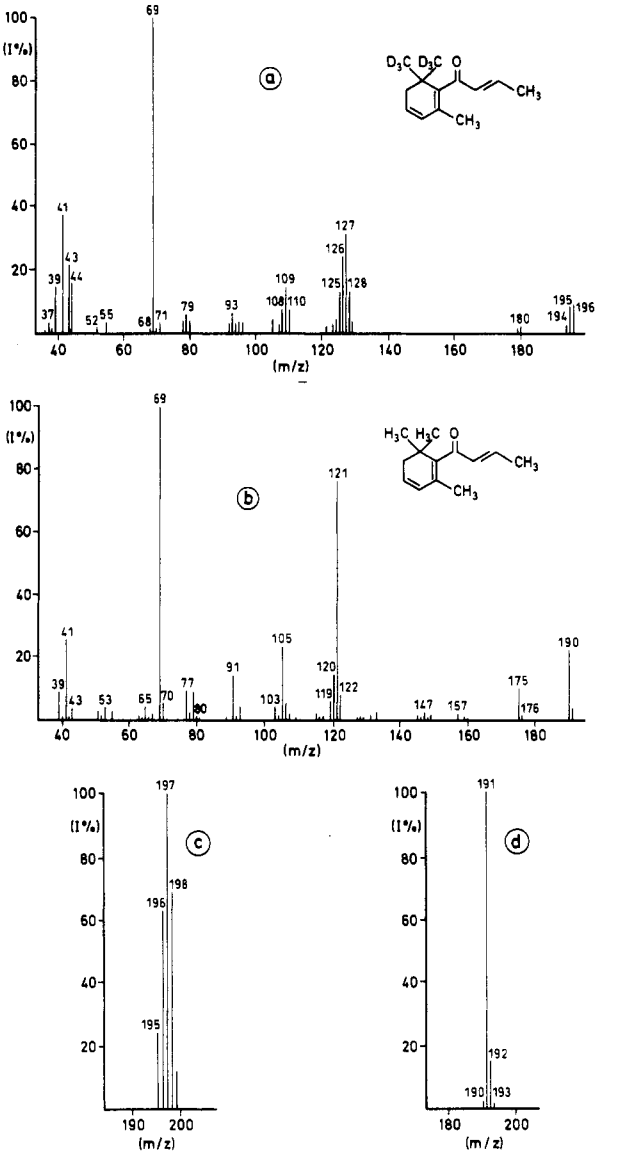

Figure 2. Mass spectra of (a) [^{2}H]-β-damascenone (MS-EI), (b) β-damascenone (MS-EI), (c) [^{2}H]-β-damascenone (MS-CI), and (d) β-damascenone (MS-CI).

RESULTS AND DISCUSSION

Comparison of MS Data. Figure 2, parts a and b, indicates that the abundances of the molecular ions (m/z 190 and 196) were small in the MS-EI of the labeled and unlabeled β-damascenones. The MS-CI of these compounds was measured. The spectra obtained (Figure 2c,d) show that, in contrast to the protonated molecular ion of the unlabeled β-damascenone (m/z 191), that of [^{2}H]-β-damascenone appeared as a cluster of ions with different ^{2}H content. In this cluster the ion m/z 197, which was 6 mass units higher than the protonated molecular ion of unlabeled β-damascenone, appeared with the highest abundance (Figure 2c). This confirmed, in agreement with the synthetic route, the incorporation of six ^{2}H atoms in the trimethylcyclohexadienyl ring of the β-damascenone. For quantification the abundances of the ions m/z 196, 197, and 198 (Figure 2c) were summed as reported under Experimental Procedures.

Analysis of a Model Mixture. A model experiment with a sucrose solution containing a known amount of β-damascenone was performed to establish whether a deuterium/protium exchange did take place during the analytical procedure. During such analysis, in fact, the

Table I. Isotope Dilution Assay of β-Damascenone in a Model Mixture

compd[a]	amt added, μg	amt measd, μg
β-damascenone	14	14

[a] A mixture of unlabeled (14 μg) and labeled (48 μg) β-damascenone dissolved in 2 mL of diethyl ether was added to a solution of 300 g of sucrose in 300 mL of water. After dilution with 1.2 L of 0.2 mol/L sodium borate buffer (pH 9.0), the β-damascenone was enriched and analyzed as reported for the honey sample (cf. Experimental Procedures).

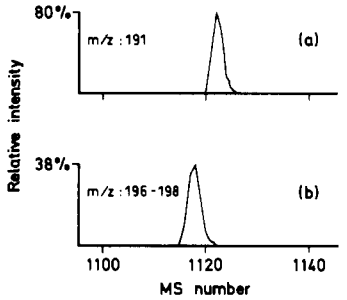

Figure 3. Analysis of the Robusta coffee sample. Mass chromatograms of (a) unlabeled β-damascenone and (b) [^{2}H]-β-damascenone.

Table II. Concentration and Odor-Activity Value (OAV) of β-Damascenone in Roasted Coffee, Black Tea, Honey, and Beer

	β-damascenone	
sample	μg/kg	log OAV[a]
Arabica coffee	260	
Robusta coffee	293	
Robusta coffee brew[b]	3.8	3.23
black tea A[c]	1.1	
black tea B[c]	1.7	
Acacia honey	3.2	3.20
Linden honey	7.8	3.59
beer	1.6	2.90

[a] The OAVs were calculated by dividing the concentration of β-damascenone by its odor threshold of 2 ng/kg (Buttery et al., 1988). [b] Brew (1 kg) obtained from roasted coffee (72 g). [c] Second-flush Darjeeling (dry material) from two plantations.

[^{2}H]damascenone added might exchange ^{2}H atoms by contact with water and with the alkaline medium at pH 9. However, the data listed in Table I indicated no difference between the theoretical value and the value measured. This indicated that no deuterium/protium exchange occurred during the analytical procedure.

Analysis of Food Samples. The analytical procedure was used to determine the β-damascenone in roasted Arabica and Robusta coffee samples. The mass chromatograms recorded for the quantification of the β-damascenone in the Arabica coffee are shown in Figure 3 as an example. The relative high amount of 260 μg/kg of the compound was calculated from the areas of the peaks with the aid of the calibration factor R.

The summary of the results in Table II reveals that the Robusta coffee sample contained approximately 10% more β-damascenone than the Arabica coffee. However, when a coffee brew was prepared from the Robusta coffee, only 18% of the β-damascenone was extracted by the hot water. Compared to roasted coffee, the two samples of black tea contained very low levels of β-damascenone.

Odor-activity values (OAVs) were calculated for β-damascenone in the honey and beverage samples (Table II). As the Linden honey contained more than twice the level of β-damascenone as did the Acacia honey, its OAV was comparatively higher. This agreed with the overall flavor impression, which was more intense in the Linden than in the Acacia honey. On the basis of the low odor threshold, the OAV of β-damascenone in beer was high, although its concentration amounted only to 1.6 μg/kg.

Conclusion. The results show that the method developed is indeed effective for a precise quantification of parts per billion levels of β-damascenone in different types of foods and beverages.

ACKNOWLEDGMENT

The work was supported by the AIF (Köln) and the Forschungskreis der Ernährungsindustrie (Hannover).

LITERATURE CITED

Acree, T. E.; Braell, P.; Butts, R. M. The presence of damascenone in cultivars of *Vitis vinifera* (Linneaus), *rotundifolia* (Michaux), and *labruscana* (Baily). *J. Agric. Food Chem.* **1981**, *29*, 688–690.

Blank, I.; Fischer, K.-H.; Grosch, W. Intensive neutral odourants of Linden honey. *Z. Lebensm. Unters. Forsch.* **1989**, *189*, 426–433.

Büchi, G.; Wüest, H. Synthetic studies on damascenones. *Helv. Chim. Acta* **1971**, *54*, 1767–1776.

Buttery, R. G.; Teranishi, R.; Ling, L. C. Identification of damascenone in tomato volatiles. *Chem. Ind. (London)* **1988**, 238.

Demole, E.; Enggist, P.; Säuberli, U.; Stoll, M.; Kovats, E. *Helv. Chim. Acta* **1970**, *53*, 541–551.

Esterbauer, H. *Fette, Seifen, Anstrichm.* **1968**, *70*, 1–4.

Kazi, M. A.; Khan, I. H.; Khan, M. Y. Alkylation of ethyl 2,6,6-trimethyl-4-oxocyclohex-2-enecarboxylate. *J. Chem. Soc.* **1964**, 1511–1512.

Kovats, E. Composition of essential oils. Part 7. Bulgarian oil of rose (*Rosa damascena* Mill.). *J. Chromatogr.* **1987**, *406*, 185–222.

Maarse, H.; Visscher, C. A. *Volatile Compounds in Food*, Suppl. 5; Division for Nutrition and Food Research, TNO: Zeist, The Netherlands, 1988.

Nursten, H. D.; Woolfe, M. L. An examination of the volatile compounds present in cooked Bramley's Seeding apples and the changes they undergo on processing. *J. Sci. Food Agric.* **1972**, *23*, 803–822.

Renold, W.; Näf-Müller, R.; Keller, U.; Wilhalm, B.; Ohloff, G. An investigation of the tea aroma. Part I. New volatile black tea constituents. *Helv. Chim. Acta* **1974**, *57*, 1301–1308.

Schieberle, P.; Grosch, W. Quantitative analysis of aroma compounds in wheat and rye bread crusts using a stable isotope dilution assay. *J. Agric. Food Chem.* **1987**, *35*, 252–257.

Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797–800.

Schreier, P.; Drawert, F. *Z. Lebensm. Unters. Forsch.* **1974**, *154*, 273–278.

Spadone, J.-C.; Takeoka, G.; Liardon, R. Analytical investigation of Rio off-flavor in green coffee. *J. Agric. Food Chem.* **1990**, *38*, 226–233.

Tressl, R.; Friese, L.; Fendesack, F.; Köppler, H. Gas chromatographic-mass spectrometric investigation of hop aroma constituents in beer. *J. Agric. Food Chem.* **1978**, *26*, 1422–1426.

Received for review May 22, 1990. Accepted August 29, 1990.

Registry No. Allylidenetriphenylphosphorane, 15935-94-1; ethyl α-isopropylideneacetoacetate, 35044-52-1; ethyl α-safranate, 35044-57-6; ethyl β-safranate, 35044-59-8; allyllithium, 3052-45-7; [^{2}H]-β-damascenone, 131619-08-4; [^{2}H]ethyl α-isopropylideneacetoacetate, 35044-52-1; deuterated acetone, 666-52-4; ethyl acetoacetate, 141-97-9; β-damascenone, 23726-93-4.