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ARTICLE

Identification of (*E,E*)-2,4-Undecadienal from Coriander (*Coriandrum sativum* L.) as a Highly Effective Deodorant Compound against the Offensive Odor of Porcine Large Intestine

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The leaves of coriander (*Coriandrum sativum* L.) exhibited a strong deodorizing effect against porcine internal organs (large intestine). The effective deodorizing compounds of coriander were identified by separating the volatile component of coriander, testing the effectiveness of each fraction against the offensive odor of porcine large intestine, and then identifying the compounds by GC-MS. The volatile component of coriander was first separated into six fractions (A−F) by preparative gas chromatography, and the deodorizing activity of each of these fractions against the offensive odor was measured. Fraction D, which showed the strongest deodorizing effect, was then separated into 12 subfractions by preparative GC. The deodorant activity of each subfraction was evaluated, and the deodorant compounds were identified by GC-MS. It was discovered that (*E,E*)-2,4-undecadienal was the most effective deodorizing compound. The deodorizing activity of (*E,E*)-2,4-undecadienal on the porcine large intestine increased as with concentration, reaching almost complete deodorizing ability at 10 ppb.

KEYWORDS: Coriander; deodorizing; (*E,E*)-2,4-undecadienal; offensive odor

INTRODUCTION

Coriander (*Coriandrum sativum* L.) is a hardy annual member of the Umbelliferae family indigenous to the Near East and Mediterranean regions. The fresh green leaves of the plant, commonly known as cilantro or Chinese parsley, also have wide culinary use in the cuisines of China, Mexico, South America, India, and South Asia to add flavor to foods or to hide the unpleasant smell of certain cooking materials. The seeds have a refreshing aroma and sweet taste.

One of the important attributes of coriander is its aroma; this quality, which is paramount to the culinary value of the fresh herb, usually decreases before the visual quality decreases (*1*). Many volatile and aromatic compounds have been isolated from coriander (*2*), and there is much information available on the identification of coriander fruit essential oils (*3*−*5*). Recently, studies have been conducted to assess the functionality of the volatile compounds of coriander leaves, such as bactericidal effects against *Salmonella choleraesuis* microorganisms (*6*).

Studies have also been conducted on the deodorizing effects of extracted solutions and essential oils of coriander and various other plants including the perilla family, the rose family, the camellia family, bergamot, orange, thyme, sage, and rosemary (*7*−*11*). For example, 4′-hydroxy-5,5′-diisopropyl-2,2′-dimethylbiphenyl-3,4′-dione is a deodorizing component from thyme (*12*), and 2,6-dimethoxy-1,4-denzoquinone is a deodorizing compound from hydrangea (*13*).

In a previous study we showed that coriander leaves with stems could exert a deodorizing effect against the offensive odor of the porcine large intestine and that this deodorizing effect was higher than that of other herbs, wild grasses, and green tea (*14*). GC−olfactory, GC-MS, and aroma extract dilution analysis methods detected four main compounds contributing to the porcine large intestine odor. However, these components of the porcine large intestine were not decomposed by coriander despite its remarkable deodorant effect upon the offensive odor (*14*). Therefore, the deodorizing compound of coriander leaves and stem against this offensive odor has not yet been identified, and so the aim of the current study was to isolate and identify the deodorizing compounds, effective against the offensive odor of the porcine large intestine, from coriander leaves and stems.

MATERIALS AND METHODS

Materials. Coriander (*C. sativum* L.), from Sakata Seed Co., Ltd., Kanagawa, Japan, was cultured in a greenhouse at Hiroshima Prefectural University from March 10 through July 10, 2001. The leaves with stems were sampled at the seedling stage (40 days after sowing), frozen with

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liquid nitrogen, crushed, and stored at −80 °C until the time of extraction of volatile compounds. The porcine large intestine used was from a pig slaughtered in Fukuyama City, Hiroshima, Japan. The porcine colon was immediately frozen and transported to our laboratory, then cut into minced meat, and stored at −80 °C.

Chemicals. (*E*,*E*)-2,4-Undecadienal (95.0%) was purchased from Bedoukian Research, Inc. (*E*)-2-Undecenol (98.0%) and 2-phenylethanol (98.0%) were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Extraction of Coriander Volatile Compounds. Volatile components were extracted from coriander using the Porapak Q method (PQM) as described by Maneerat et al. (*15*). Crushed coriander samples of 10 g of fresh weight (FW) were added to 200 mL of distilled water, and the resulting mixture was stirred overnight at room temperature. Then the samples were centrifuged at 12000*g* for 20 min, and the supernatant was filtered with a glass filter under reduced pressure. At room temperature, the extract was then poured into a PQ column (i.d. 2 × 40 cm) to adsorb the volatile compounds on the PQ resin, and the column was washed with 100 mL of distilled water. The volatile compounds were then dissolved in 100 mL of diethyl ether to elute the absorbed compounds. Ten microliters of 1% cyclohexanol was added as an internal standard, and then the eluate containing the volatile compounds was dried over hydrous sodium sulfate overnight at room temperature and concentrated to ∼100 μL under a nitrogen stream. Samples of 1 or 2 μL of the extracts were drawn for gas chromatograph (GC) analysis, preparative GC analysis, or GC−mass spectrometry (GC-MS) analysis.

Preparative GC Analysis. Preparative GC analysis was performed with a Shimadzu gas chromatograph model 14A (Shimazu Co., Ltd., Kyoto, Japan), equipped with a Supelco-WAX capillary column (0.75 mm i.d. × 60 m, membrane pressure 1.0 m, J&W Scientific Corp., Folsom, CA) and a flame ionization detector (FID). The capillary column was maintained at 40 °C for 10 min after injection, then heated at 3 °C min^{-1} to 220 °C, and maintained at that temperature for 30 min. The helium carrier gas flow rate was 56 cm s^{-1} with splitless injection. The injector and detector temperatures were 230 and 250 °C, respectively. Thirty centimeter shot capillaries (0.53 mm i.d. × 25 cm, 2.0 μm, GL Science Co., Ltd., Tokyo, Japan) were used to trap separated fractions or compounds with a split ratio of 20:1. The separated compounds trapped in shot capillaries were eluted with a drop of 100 μL of diethyl ether from the end of shot capillaries using a microsyringe. Preparative GC was repeated until concentration proper of each compound. The eluate of each faction was dropped on filter paper, and then the deodorant activity test was conducted.

GC Analysis. GC analysis was performed with a Shimadzu GC-17A with an FID and a Shimadzu Chromatopac C-R7A integrator (Shimadzu Co., Ltd.). The compounds were separated on a 30 m × 0.25 mm (i.d.) fused silica capillary column coated with a 0.25 μm film bonded polar DB-WAX (J&W Scientific Inc.). The column temperature program was the same as that used in the preparative GC analysis. Open split injection was conducted with a split ratio of 1: 20, and helium was used as the carrier gas at 30 cm s^{-1}. The injector and detector temperatures were 230 and 250 °C, respectively.

GC−Mass Spectrometry (GC-MS) Analysis. GC-MS was performed with a Shimadzu GC-17A gas chromatograph coupled with a Shimadzu QP5050 mass spectrometer (Shimadzu Co., Ltd.). The column (DB-WAX, 60 m × 0.25 mm i.d. ; J&W Scientific Inc.) and temperature program were the same as those used for the GC analysis. Split injection was performed with helium as the carrier gas. Compounds were identified by the use of NIST and the injection of pure compounds and a comparison of their retention times (as Kovats index).

Deodorizing Activity Test. The offensive odor samples (2.0 g of the porcine internal organs) were placed in a 50 mL conical beaker and heated in water bath (60 °C) for 10 min. Then, 20 μL of 100 ppb (*E*)-2-undecenol, 100 ppb 2-phenylethanol, and 0.01−100 ppb (*E*,*E*)-2,4-undecadienal solution was dropped on filter paper and added to 2.0 g of porcine large intestine in a beaker. Two nanograms of (*E*)-2-undecenol and 2-phenylethanol was dissolved in 20 μL of diethyl ether, respectively. The odorant solutions were finally adjusted to 0.1 ng μL^{-1} (100 ppb). Amounts of 2, 20, and 200 pg and 2 ng of (*E*,*E*)-2,4-undecadienal were dissolved in 20 μL of diethyl ether. (*E*,*E*)-2,4-Undecadienal solution was finally adjusted to 1, 10, 100, and 1000 pg μL^{-1} (0.1, 1, 10, and 100 ppb). The odor intensity of the porcine internal intestine was sniffed by 10 trained members (10 healthy adults, 4 men and 6 women, ranging in age from 21 to 47 years recruited from Hiroshima Prefectural University, all nonsmokers). The members then graded the deodorizing activity against the offensive odor, that is, the odor intensity, using a 5-point scale: perfectly deodorized, 0; almost deodorized, 1; considerably deodorized, 2; slightly deodorized, 3; not deodorized at all, 4.

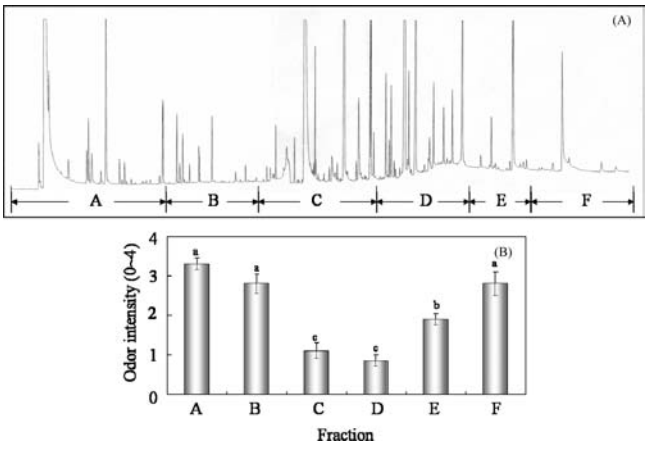

Figure 1. Chromatogram of the volatile compounds of coriander PQ extraction (**A**) and deodorizing activity of preparative GC fractions (A−F) of coriander PQ absorbent against porcine large intestine (**B**). The volatile compounds were grouped into six fractions (A−F) using shot capillaries with the following retention times: A, 0.0−32.0 min; B, 32.1−48.0 min; C, 48.1−60.0 min; D, 60.1−72.0 min; E, 72.1−80.0 min; F, 80.1−100 min. Deodorization intensity: 0, perfectly deodorized; 1, almost deodorized; 2, considerably deodorized; 3, a little deodorized; 4, completely not deodorized. Standard errors are based on three replicate samples from each of 10 people. Fractions with the same letters are not significantly different at $p < 0.05$ by Tukey−Kramer test.

RESULTS AND DISCUSSION

Figure 1A shows the chromatic separation of about 380 volatile compounds in the coriander PQ extraction, separated into 6 fractions (A−F) by preparative GC. The odor intensities of fractions A−F were 3.3, 2.8, 1.1, 0.9, 1.9, and 2.8, respectively (**Figure 1B**), indicating that fractions D and C had the highest deodorizing effects.

Fraction D was then further separated into four subfractions (α−δ) by preparative GC (**Figure 2A**). The odor intensity scores of the subfractions were 2.4, 0.9, 2.5, and 2.6, respectively, indicating that subfraction β exhibited the strongest deodorizing effect on the offensive odor of porcine large intestine (**Figure 2B**). Finally, the β subfraction was separated into three further fractions (β-1−3) by preparative GC. The odor intensity scores ranged from 3.0 for β-1 and β-3 to 0.9 for β-2, indicating that the β-2 fraction had the strongest deodorizing effect on the offensive odor of porcine large intestine (**Figure 2C**).

Further detailed GC-MS analysis of the β-2 fraction showed it comprised four volatile components, (*E*)-2-undecenol, 2-phenylethanol, (*E*,*E*)-2,4-undecadienal, and an unknown compound (**Figure 3A**). Although there have been reports about the volatile compounds of coriander leaf, neither the essential oils and seed oils (*16*, *17*) nor (*E*)-2-undecenol, 2-phenylethanol, and (*E*,*E*)-2,4-undecadienal have been isolated. Therefore, this study is the first to identify these compounds. (*E*)-2-Undecenol has a sweet and fruity aroma, 2-phenylethanol, which is the main compound in lilac, has a flowery aroma (*18*), and (*E*,*E*)-2,4-undecadienal has a roasted oily aroma (*19*). The identification of these compounds proves that coriander leaf has not only a green odor but also fruity and flowery odors.

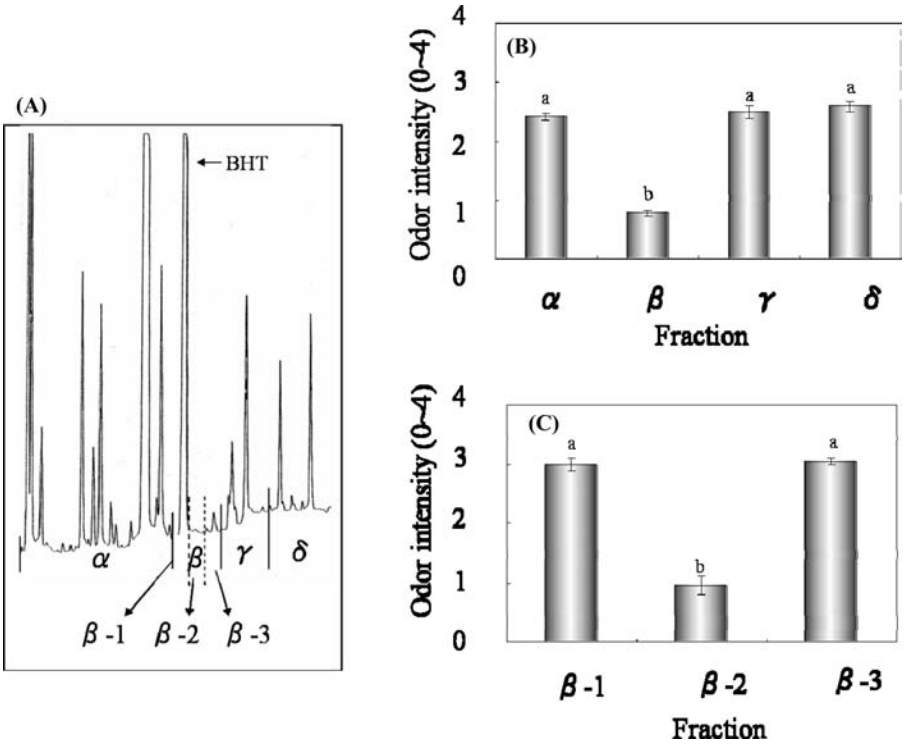

Figure 2. Expanded chromatogram of the D fraction showing subfractions α−δ (**A**). The deodorizing activity of the preparative GC subfractions (α−δ) and the further separated fractions (β-1−3) of coriander PQ absorbent against porcine large intestine are shown in panels **B** and **C**, respectively. The volatile compounds in fraction D were further separated into the subfractions using shot capillaries with the following retention times: α, 47.98−52.85 min; β, 52.86−55.87 min; γ, 55.89−58.87 min; δ, 57.88−60.30 min; β-1, 52.86−53.85 min; β-2, 53.86−54.85 min; β-3, 54.86−55.87 min. Deodorization intensity: 0, perfectly deodorized; 1, almost deodorized; 2, considerably deodorized; 3, a little deodorized; 4, completely not deodorized. Standard errors are based on three replicate samples from each of 10 people. Fractions with the same letters are not significantly different at $p < 0.05$ by Tukey−Kramer test. BHT, 2,6-bis(1,1-dimethylethyl)-4-methylphenol.

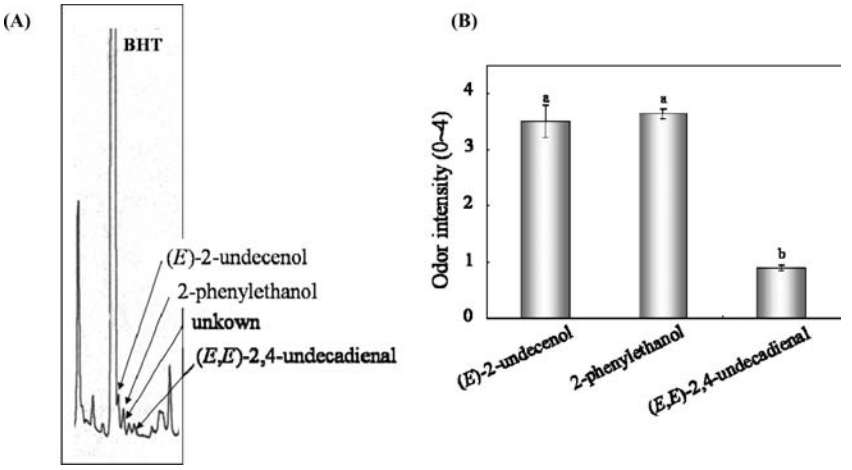

Figure 3. Chromatogram of four compounds contained in β-2 fraction (**A**) and the deodorization effect of standard compounds of (*E*)-2-undecenol, 2-phenylethanol, and (*E*,*E*)-2,4-undecadienal on a porcine large intestine smell (**B**). Deodorization intensity: 0, perfectly deodorized; 1, almost deodorized; 2, considerably deodorized; 3, a little deodorized; 4, completely not deodorized. The deodorization activity test was carried out with 2.0 g of porcine large intestine immediately after the addition of 100 ppb standard compound solutions of (*E*)-2-undecenol, 2-phenylethanol, and (*E*,*E*)-2,4-undecadienal drops on filter paper. Standard errors are based on three replicate samples from each of 10 people. Fractions with the same letters are not significantly different at $p < 0.05$ by Tukey−Kramer test.

The deodorizing activity of (*E*)-2-undecenol, 2-phenylethanol, and (*E*,*E*)-2,4-undecadienal against the porcine large intestine odor is shown in **Figure 3B**. The odor intensities of (*E*)-2-undecenol-, 2-phenylethanol-, and (*E*,*E*)-2,4-undecadienal-treated porcine intestine samples were 3.5, 3.6, and 0.8, respectively, indicating that the deodorizing effect of (*E*,*E*)-2,4-undecadienal was as strong as that of the entire β-2 fraction (**Figure 3B**). This finding indicated that (*E*,*E*)-2,4-undecadienal is an effective deodorizing compound against porcine large intestine. Moreover, the deodorizing effect of (*E*,*E*)-2,4-undecadienal against 2.0 g of porcine large intestine was dramatically elevated when the treatment amount of (*E*,*E*)-2,4-undecadienal was increased from 0.1 to 100 ppb (**Figure 4**). Although (*E*,*E*)-2,4-undecadienal has a fatty and green odor, at low concentrations of 0.1−100 ppb it is

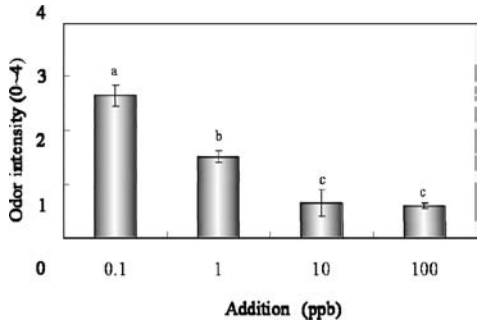

Figure 4. Deodorization effect of (*E*,*E*)-2,4-undecadienal on a porcine large intestine smell. Deodorization intensity: 0, perfectly deodorized; 1, almost deodorized; 2, considerably deodorized; 3, a little deodorized; 4, completely not deodorized. An amount of 0.1 or 1.0 ppm (*E*,*E*)-2,4-undecadienal was dropped on a filter paper in a beaker and diluted to 2−20 μL to obtain final (*E*,*E*)-2,4-undecadienal concentrations of 0.01−100.00 ppb. These solutions were tested against 2.0 g of porcine large intestine. Standard errors are based on three replicate samples from each of 10 people. Concentrations with the same letters are not significantly different at $p < 0.05$ by Tukey−Kramer test.

almost odorless. Thus, it is possible that the deodorizing effect of (*E*,*E*)-2,4-undecadienal against porcine large intestine might not be caused by a masking effect, in which the offensive odor is hidden by another strong odor. However, in a previous study, Kohara et al. reported five main compounds contributing to the porcine large intestine odor, including 4-methylphenol (sludge-like odor), unknown compound I (sludge-like odor), unknown compound II (porcine large intestine-like odor), indole (excrement-like odor), and skatole (excrement-like odor) by GC−Olfactory, GC-MS, and the aroma extract dilution analysis methods (*20*). These components of the porcine large intestine were not decomposed by coriander, despite its remarkable deodorant effect on the offensive odor. The authors suggested that there is a very strong possibility that the observed deodorizing effect in their study was not due to decomposition or reduction of the offensive odor compounds, but could be explained by the masking or the modification phenomenon (*21*). As for the deodorization mechanism, four types of mechanisms, chemical, physical, sensuous, and biological deodorant activities, are reported (*22*). In this experiment, the deodorant mechanism of the coriander was presumed that the possibility that was not a chemical, physical deodorant it but sense deodorant was high, whereas the compound did not decompose those offensive odors. Therefore, their conclusions differ from those in our current study. To establish the deodorizing mechanism of coriander, it will be necessary to establish the exact deodorizing mechanism of (*E*,*E*)-2,4-undecadienal.

In this work, the volatile component of the leaves of coriander was separated into fractions, and we identified (*E*,*E*)-2,4-undecadienal as having a strong deodorizing effect against the offensive odor of porcine large intestine. However, the exact deodorizing mechanism remains to be clarified.

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