Identification of odor-active trace compounds in Damask rose (*Rosa damascena*)

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Abstract

The flower scent of Damask rose (*Rosa damascena*) was investigated. Two ultratrace components that exhibited high flavor dilution factors were detected as odor-active compounds by aroma extract dilution analysis (AEDA). One of the trace compounds with a woody note was identified as rotundone by multidimensional gas chromatography–mass spectrometry/olfactometry (MD-GC-MS/O), whereas the other with a citrus note was identified as 4-(4-methyl-3-pentenyl)-2(5*H*)-furanone (MPF) via fractionation of a commercial rose absolute from *R. damascena*. To the best of our knowledge, this is the first study that reflects the organoleptic importance of these two compounds for the rose scent. Sensory analyses were performed to assess the effects of rotundone and MPF. Results revealed that the addition of 50 μg/kg rotundone and 5 μg/kg MPF to the aroma reconstitute of *R. damascena* provided blooming and natural aspects to it. In addition, the presence of rotundone and MPF in five types of fragrant roses was examined. MPF was also detected in fruits (e.g., lemon, orange, grapefruit, apple, and Muscat grape), black tea, and beer.

Introduction

The rose scent is crucial for flavors and fragrances. In particular, the rose note is essential for floral perfume compositions. Among the large varieties and forms of roses, Damask rose (*Rosa damascena*) is one of the main species of roses that are cultivated in the fragrance industry. The typical aroma concentrates used for fragrance products include rose oil, rose water, rose absolute, and rose concrete. Nevertheless, the aroma of these processed products is different from that of natural rose flowers. Furthermore, the rose aroma reconstitutes with chemical compounds are different from those of natural rose flowers, thus possibly suggesting that the remaining unknown components are the key to the secret of the rose scent. Volatile compounds of natural rose products have been extensively analyzed for many years; however, not many studies have reported the headspace aroma of natural rose flowers. Therefore, this study aims to sensorically characterize and identify the main odorants that are present in the aroma concentrate of the headspace volatiles of *R. damascena* by aroma extract dilution analysis (AEDA), and identify the compounds that differentiate the scent of natural rose from that of artificial rose aroma reconstitutes.

Experimental

Materials

The petals of *R. damascena* were handpicked from the garden of the T. Hasegawa R&D center in the morning. Absolute from *R. damascena* was purchased from Biorandes Co., (Le Sen, France).

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Dynamic headspace analysis and AEDA

First, immediately after picking the petals of *R. damascena* (42.6 g), they were placed into a glass chamber. A constant flow rate of 1.5 L/min was used with air entering the chamber through a charcoal filter and leaving the chamber via passage through 2.0 g of a Tenax TA 60/80 adsorbent (GL Sciences Co., Tokyo, Japan). After collecting the volatiles for 6 h, 20 mL of pentane and 20 mL of diethyl ether were used for elution. The eluent was collected and concentrated to ca. 100 µL via solvent distillation using a Vigreux column at 43 °C. The concentrate was subjected to GC-MS/FID and GC-O analyses equipped with a polar column (InertCap WAX). In addition, MD-GC-MS/O analysis (first column: InertCap WAX, second column: InertCap 1MS) was performed to elucidate the two unknown compounds. For AEDA [1], the concentrated volatile was diluted stepwise with diethyl ether to obtain dilutions of 1:5, 1:25, 1:125, 1:625, etc.

Elucidation of the citrus-like odor compound

The absolute from *R. damascena* (330 g) was fractionated by distillation, silica-gel column chromatography, and two-step high-performance liquid chromatography (HPLC). GC-O analysis was performed to confirm the presence of the target citrus-like odor compound, thus finally obtaining the HPLC fraction (2.8 mg). The chemical structure of the target compound was assumed from high-resolution mass and NMR spectra. Identification was further confirmed by matching the analytical data and odor qualities of the isolated citrus-like odor compound with those of the estimated compound synthesized according to a previously reported method [2].

Threshold measurement of MPF

The odor threshold of MPF in water was determined according to a previously reported method [3]. Panelists [n = 23 (16 males and 7 females; age range 20–60 years)] were employees of the R&D Center of T. Hasegawa Co., Ltd. Assessments were conducted orthonasally. Panelists also simultaneously evaluated the odor of the sample that they had successfully recognized.

Evaluation of the effect of rotundone and MPF

The triangle test was performed to assess the effects of rotundone and MPF on the rose aroma reconstitutes of *R. damascena*. Four aroma reconstitutes (samples A–D) were evaluated. Sample A comprised an aroma reconstitute of *R. damascena* diluted in dipropylene glycol at 5% w/w. Samples B, C, and D comprised fragrance solutions with the same aroma reconstitute with rotundone (50 µg/kg), MPF (5 µg/kg), and rotundone (50 µg/kg) and MPF (5 µg/kg), respectively.

Identification of rotundone and MPF in various types of roses and foods

The headspace gases of the living flowers of *Rosa centifolia*, “Neige Perfum,” “Pope John Paul II,” “Lady Hilingdon,” and “Grand Mogul” were pumped to pass through Tenax TA. The adsorbents were eluted with pentane and diethyl ether, followed by concentration. The concentrates were subjected to MD-GC-MS/O analysis to tentatively identify rotundone and MPF.

Cold-pressed oils of lemon, orange, grapefruit, and distilled oil of lime were purchased. The aroma concentrates of apple, Muscat grape, black tea, and beer were prepared by solvent extraction and solvent-assisted flavor evaporation method [4]. The above-mentioned samples were subjected to MD-GC-MS/O analysis to tentatively identify MPF.

Results and discussion

Dynamic headspace analysis and AEDA

Dynamic headspace sampling was employed to prepare the headspace aroma concentrates of *R. damascena*. From the AEDA results, along with the major compounds such as 2-phenylethanol, geraniol, and citronellol, two ultratrace components with a high flavor dilution factor (FD 625) were detected as odor-active compounds. One trace compound was identified as rotundone, with a woody note (Figure 1) by MD-GC-MS/O analysis with the same retention time, MS spectrum, and odor qualities as those of the authentic synthesized rotundone. However, the other (citrus note) was detected at sufficiently low trace levels such that its structure could not be identified. Rotundone has been identified as an odor-active component in patchouli oil [5], frankincense oil [6], Shiraz wine [7], peppers [7], and several fruits [8]. To the best of our knowledge, rotundone has not been detected in roses.

Elucidation of the citrus-like odor compound

The citrus-like odor compound was detected in commercially available rose absolute from *R. damascena*. Therefore, the target citrus-like compound was isolated from rose absolute by distillation, silica-gel column chromatography, and two-step HPLC. As confirmed by GC-FID analysis, the final purity of the isolated compound was 97%. This compound was assumed to be 4-(4-methyl-3-pentenyl)-2(5*H*)-furanone (MPF; Figure 1) from high-resolution mass spectra and NMR spectra. Identification was further confirmed by matching the analytical data and odor qualities of the isolated MPF with those of synthesized MPF. MPF has been identified in rose oil [9] as well as in the secretion of acarid mites [10]. However, to the best of our knowledge, this is the first study that reports on the chemosensory properties of MPF. In the threshold measurement of MPF, panelists have described that MPF emits a citrus-like (lemon, orange, and grapefruit) and floral odor (muguet and jasmine) with a fairly low threshold of 3.6 µg/kg in water.

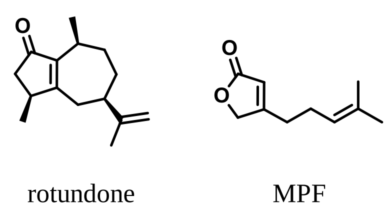

Figure 1: Chemical structures of the elucidated odor-active trace compounds from the headspace aroma of rose petals.

Effects of rotundone and MPF

The triangle test was performed to examine the effects of rotundone and MPF on the rose aroma reconstitutes of *R. damascena.* Figure 2 shows the results of this test. Sample B and C were not significantly distinguished from sample A. Only sample D was significantly distinguished from sample A. Moreover, panelists who could distinguish sample D from sample A evaluated the aroma of sample D as “more blooming than A” or “more natural than A.” These results suggested that the aroma of the rose aroma reconstitute with the addition of rotundone and MPF was more similar to that of a natural rose flower. Interestingly, the panelists did not differentiate samples A and D as “woody” or “citrus-like.” The effect of these two added compounds is expected to differentiate the aroma of natural rose from that of the artificial rose aroma reconstitutes.

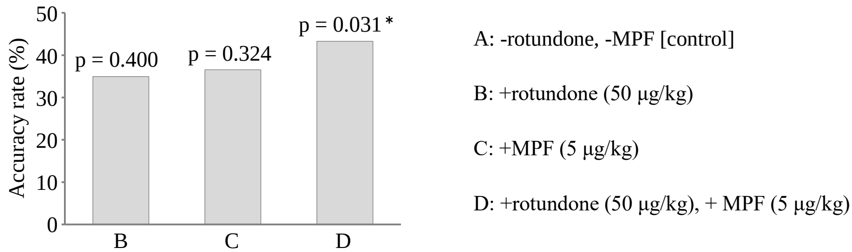

Figure 2: Accuracy rate of the triangle test (Identification of sample B, C, or D against A). n = 52, *Binominal test, $p < 0.05$

Identification of rotundone and MPF in various types of roses and foods

MD-GC-MS/O analysis was employed to examine the presence of rotundone and MPF in five types of fragrant roses. The former was detected in all roses, while the latter was detected in three roses (i.e., *Rosa centifolia*, "Neige Perfum," and "Pope John Paul II," respectively). Rotundone has been identified in several fruits as a potent odor-active compound [8]; therefore, we examined the presence of MPF in fruits. The results revealed that MPF was detected in lemon, orange, grapefruit, apple, and Muscat grape. In addition, MPF was also detected in black tea and beer. These results indicated that MPF is widely distributed not only in roses but also in various foods. To confirm the contribution of MPF to the aromas of natural resources, quantitative studies need to be performed.

References

1. Grosch, W. (1994) Flavour Frag. J. 9: 147-158.
2. Poulter, C. D., Wiggins, P. L. and Plummer, T. L. (1981) J. Org. Chem. 46: 1532-1538.
3. Meilgaard, M., Civille, G. V. and Carr, B. T. (2016) Sensory Evaluation Techniques (5th ed) CRC Press: Boca Raton, FL, pp. 153-163.
4. Engel, W., Bahr, W. and Schieberle, P. (1999) Eur. Food Res. Technol. 209: 237-241.
5. Kaiser, R. (2006) In: Meaningful Scents around the World. Olfactory, Chemical, Biological and Cultural Considerations (Kisakürek, M. V., ed.) Verlag Helvetica Chimica Acta/Wiley-VCH, pp 59−64.
6. Niebler, J., Zhuravlova, K., Minceva, M. and Buettner, A. (2016) J. Nat. Prod. 79: 1160-1164.
7. Wood, C., Siebert, T. E., Parker, M., Capone, D. L., Elsey, G. M., Pollnitz, A. P., Eggers, M., Meier, M., Vössing, T., Widder, S., Krammer, G., Sefton, M. A. and Herderich, M. J. (2008) J. Agric. Food Chem. 56: 3738-3744.
8. Nakanishi, A., Fukushima, Y., Miyazawa, N., Yoshikawa, K., Maeda, T. and Kurobayashi, Y. (2017) J. Agric. Food Chem. 65: 4464-4471.
9. Ohloff G. and Demole E. J. (1987) Chromatog. 406: 181-183.
10. Tarui H., Mori N., Nishida R., Okabe K. and Kuwahara Y. (2002) Biosci. Biotechnol. Biochem. 66: 135-140.