Chemistry and Biology of Aroma and Taste

Identification of Odor-active Trace Compounds in Blooming Flower of Damask Rose (*Rosa damascena*)

Teruhisa Ohashi, Yamato Miyazawa, Susumu Ishizaki, Yoshiko Kurobayashi, and Tsukasa Saito

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.9b03391 • Publication Date (Web): 12 Jun 2019

Identification of Odor-active Trace Compounds in Blooming Flower of Damask Rose (*Rosa damascena*)

Teruhisa Ohashi, Yamato Miyazawa, Susumu Ishizaki, Yoshiko Kurobayashi, Tsukasa Saito*

Research & Development Center, T. Hasegawa Co., Ltd., 29-7, Kariyado, Nakahara-ku, Kawasaki-shi, 211-0022, Japan

AUTHOR ADDRESS: teruhisa_ohashi@t-hasegawa.co.jp

RUNNING HEAD

Odor-active Trace Compounds in Damask Rose (*Rosa damascena*)

*Teruhisa Ohashi FOOTNOTE.

TEL: +81-44-411-0298; FAX: +81-44-434-5257; E-mail: teruhisa_ohashi@t-hasegawa.co.jp

ABSTRACT

The flower scent of the damask rose (*Rosa damascena*) was studied. Two ultra-trace components exhibiting high flavor dilution factors were detected as odor-active compounds via aroma extract dilution analysis (AEDA). One of these had a woody note and was identified as rotundone using multidimensional gas chromatography–mass spectrometry–olfactometry (MD-GC-MS-O), while the other had a citrus note and was identified as 4-(4-methylpent-3-en-1-yl)-2(5*H*)-furanone (MPF) by fractionation of a commercial rose absolute from *R. damascena*. To the best of our knowledge, this is the first study addressing the organoleptic importance of these two compounds in the rose scent. Sensory analysis was conducted to evaluate the effects of rotundone and MPF. Adding 50 μg/kg rotundone and 5 μg/kg MPF to the aroma reconstitute of *R. damascena* provided it with blooming and natural characteristics. Additionally, the existence of rotundone and MPF in five types of fragrant roses was investigated.

KEYWORDS: rose; *Rosa damascena*; odor-active compound; AEDA; isolation

INTRODUCTION

The rose scent is essential to flavors and fragrances. Particularly, the rose note is key to floral perfume compositions. Typical aroma concentrates employed in fragrance products are rose oil, rose water, rose absolute, and rose concrete. Nonetheless, the aromas of these processed products differ from those of natural blooming rose flowers due to possible heating or oxidation during the manufacturing processes. Furthermore, the scent of rose cannot be imitated by merely using known chemicals, indicating that the remaining unknown components are key to the enigma of the rose scent. Among the large rose varieties and forms, the damask rose (*Rosa damascena*) is one of the main species cultivated for the fragrance industry[1]. Volatile compounds of natural rose products from *R. damascena* have been comprehensively analyzed for many years.[2,3] In these studies, very small amount of constituents, such as rose oxides[4] and rose ketones[5,6], were identified as key odorants of rose oil. Further, the rose oil aroma profile was recently investigated using aroma extract dilution analysis (AEDA) and sensory analysis[7]. However, few studies have reported the aroma of the blooming *R. damascena* flower. Thus, this study aims to sensorially analyze and identify the main odorants in the aroma emitted from the blooming flower of *R. damascena* and identify the compounds differentiating the natural rose scent from that of artificial rose aroma reconstitutes. *R. damascena* has several cultivars. We planted *R. damascena* 'trigintipetala,' which is one of the cultivars grown for rose oil production in Bulgaria,[8] and examined its beautiful flower scent in detail. To define the odor-active compounds in the volatiles of *R*. *damascena* flower, AEDA was conducted for the aroma concentrate obtained by dynamic headspace sampling. Further, follow the structural elucidation of the two odor-active unknowns present in trace. These two compounds were found to have "woody" note or "citrus-like" note and have never been regarded as important in the rose scent.

In addition, we discuss the effects of these compounds on the rose aroma reconstitutes of *R. damascena* and the existence of these compounds in five types of fragrant rose cultivars.

MATERIALS AND METHODS

Materials. Seedlings of *R. damascena* 'trigintipetala,' *Rosa centifolia* 'bullata,' *Rosa* 'Neige Parfum,' *Rosa* 'Pope John Paul II,' *Rosa* 'Lady Hilingdon,' and *Rosa* 'Grand Mogul' were obtained from nurseries in Japan and planted in the garden of the T. Hasegawa R&D Center. Absolute from *R. damascena* was obtained from Biolandes Co., (Le Sen, France).

Chemicals. Wakogel C-100 was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. All other reagents and solvents were obtained from commercial suppliers and were used without further purification. The chemicals for triangle test were obtained from T. Hasegawa (Kawasaki, Japan). Rotundone [(3*S*,5*R*,8*S*)-5-isopropenyl-3,8-dimethyl-3,4,5,6,7,8-hexahydro-1(2*H*)-azulenone] (**6**) was synthesized from (−)-guaiol in our laboratory.[9]

Synthesis of Authentic 4-(4-methylpent-3-en-1-yl)-2(5*H*)-furanone (MPF). Using a modified literature procedure,[10] 4-(4-methylpent-3-en-1-yl)-2(5*H*)-furanone (**1**) was synthesized from 3-(1-ethoxyethoxy)prop-1-yne (**2**) via ethoxycarbonylation, conjugate addition of the 4-methylpent-3-en-1-yl moiety, deprotection, and lactonization (**Figure 1**). The description of the reaction processes in Figure 1 is provided in the Supporting Information.

Dynamic Headspace (DHS) Analysis. The petals of *R. damascena* 'trigintipetala' (20 flowers; 42.6 g) were hand-picked from the garden of the T. Hasegawa R&D Center in the morning. Open flowers that emitted a strong scent were chosen. Immediately after picking, they were placed in a glass chamber. A constant flow rate of 1.5 L/min was employed, with air entering the chamber through a charcoal filter and leaving it via a passage through 2.0 g of

Tenax TA 60/80 adsorbent (GL Sciences Co., Tokyo, Japan). The volatiles were collected for 6 h at 25°C before using 20 mL each of pentane and diethyl ether for elution. The eluent was collected and concentrated to ca. 100 μL by solvent distillation using a Vigreux column at 43°C. The concentrate was subjected to GC-MS/flame ionization detector (FID) using a polar column (InertCap WAX).

Aroma Extract Dilution Analysis (AEDA). The flavor dilution (FD) factors of the odor-active compounds were determined by AEDA.[11] The concentrated volatiles of *R. damascena* were diluted stepwise with diethyl ether to obtain dilutions in the series 1:5, 1:25, 1:125, 1:625, and each dilution was analyzed via GC-O with a polar column (InertCap WAX). Two experienced assessors conducted AEDA.

Determination of the Citrus-like Odor Compound. The absolute from *R. damascena* was fractionated by distillation, silica gel column chromatography, and high-performance liquid chromatography (HPLC).

Molecular Distillation. Rose absolute from *R. damascena* (330.5 g) was distilled by employing simple molecular distillation apparatus (MDU60-05J; Asahi Glassplant Inc., Japan) under reduced pressure (90°C/2.5–0.01 kPa) to obtain a distillate (144.2 g) and residue (172.2 g). The target citrus-like odor compound was detected by GC-O. The distillate was used in the next step.

Fractional Distillation. The distillate (137.5 g) from the molecular distillation was fractionated by distillation to give distillate fractions (fraction ii-1, 85°C–90°C/0.23–0.27 kPa, 37.4 g; fraction ii-2, 99°C–104°C/0.23 kPa, 39.4 g; fraction ii-3, 110°C–115°C/0.23 kPa, 25.2 g)

and a residue (33.4 g). The target compound was identified by GC-O. The residue was used in the next step.

Silica Gel Column Chromatography. The residue (32.4 g) from the fractional distillation was further fractionated by column chromatography using silica gel (Wakogel C-100, 650 g) with the following hexane/ethyl acetate mixtures: 100:0, 30:1, 10:1, 3:1, and 0:100 (3,900 mL each). Each fraction was evaporated (fractions iii-1–iii-5) and analyzed by GC-O. A 11.1 g sample of concentrated effluent was collected from fraction iii-4, which was confirmed to contain the target aroma by GC-O.

Preparative High-Performance Liquid Chromatography (HPLC). Two successive HPLC fractionations were conducted on a concentrate of fraction iii-4 (10.7 g) obtained by silica gel column chromatography. For each HPLC fractionation, the target compound was identified by GC-O. Finally, the HPLC fraction (2.8 mg) was obtained. GC-MS-FID analysis of this fraction suggested that the main component was the target compound (97%). High-resolution mass spectrometry (HRMS) (field ionization mode) calculated for $C_{10}H_{14}O_2$ was 166.09938 (found 166.10041). The chemical structure of the target compound was deduced from HRMS and nuclear magnetic resonance (NMR) spectra. Identification was further confirmed by comparing the analytical data and odor qualities of the isolated target compound with those of synthesized authentic 4-(4-methylpent-3-en-1-yl)-2(5*H*)-furanone.

High-Performance Liquid Chromatography Fractionation. The instrument system comprised a Shimadzu LC-20AD pump, SIL-20ACHT autosampler, CTO-20AC column oven, SPD-M20A detector, CBM-20A controller, and DGU-20A5 degasser (Shimadzu Corp., Kyoto, Japan). Two successive HPLC fractionations were conducted. The initial HPLC conditions were

as follows: an Inertsil ODS-3 column (5 μm particle size, 250 mm × 14 mm i.d., GL Sciences Co., Tokyo, Japan) was connected to the instrument system. The gradient elution was as follows: 0–23 min, methanol/water = 40:60 → 63:37; 23–28 min, methanol/water = 100:0; 28–35 min, methanol/water = 40:60. The solvent flow rate was 9.3 mL/min, and the column temperature was 30°C. The effluent was monitored at λ = 220 nm, the eluent was collected between 25.0 and 25.6 min, and methanol was evaporated using a rotary evaporator from it. NaCl (25% w/w) was added to the residue before extracting with diethyl ether, and the diethyl ether layer was dried over $NaSO_4$ and concentrated in vacuo. A second HPLC fractionation was conducted on the concentrate, under the following conditions: an Inertsil ODS-3 column (5 μm particle size, 250 mm × 4.6 mm i.d., GL Sciences Co., Tokyo, Japan) was connected to the instrument system. The gradient elution was as follows: 0–18 min, acetonitrile/water = 55:45 → 73:27; 18–28 min, acetonitrile/water = 100:0; 28–40 min, acetonitrile/water = 55:45. The solvent flow rate was 1.0 mL/min, and the column temperature was 40°C. At retention times between 21.3 and 22.5 min, the eluent was collected, then was concentrated using the procedure described above.

Gas Chromatography–Mass Spectrometry–Flame Ionization Detection (GC-MS-FID). GC-MS-FID was conducted with an Agilent 7890 gas chromatograph coupled with an Agilent MSD5975 quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and an FID fitted with an InertCap WAX capillary column (0.25 mm i.d. × 60 m, 0.25 μm film thickness, GL Sciences Co.). The effluent from the column at the end of the capillary was divided into two branches and routed via deactivated fused silica capillaries to the mass spectrometer and FID. The injection port was maintained at 250°C. The split ratio was 10:1, and 1 μL of sample was injected. The oven temperature was maintained at 40°C for the first 2 min then increased to 230°C at 3°C/min, with a constant helium carrier gas flow of 1.8 mL/min. Mass

spectra in the electron impact (EI) mode were recorded at 70 eV in scan mode (*m*/*z* of 29–550). The temperature of the transfer line was 250°C. The linear retention indices (RIs) of the compounds were calculated with respect to the retention times of a homologous series of *n*-alkanes (C5–C30). The purities of the isolated or synthesized compounds [4-(4-methylpent-3-en-1-yl)-2(5*H*)-furanone] were calculated by integrating the chromatogram obtained with the FID.

Gas Chromatography–Olfactometry (GC-O). GC-O was conducted with an Agilent 7890 GC coupled with an Agilent MSD5975 quadrupole mass spectrometer and a Gerstel ODP3 sniffing port (Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany) fitted with an InertCap WAX capillary column (0.25 mm i.d. × 60 m, 0.25 μm film thickness, GL Sciences Co.). The effluent from the column at the end of the capillary was divided into two branches and routed via deactivated fused silica capillaries to the mass spectrometer and sniffing port. The sample volume, split ratio, injection temperature, oven temperature program, carrier gas, and flow rate were the same as those employed for performing the aforementioned GC-MS-FID analysis. The linear RIs of the compounds were calculated with respect to the retention times of the homologous series of *n*-alkanes (C5–C30). The GC-O analysis of the fractions from HPLC was performed by a trained panelist who was experienced in the detection of odorants using the aforementioned AEDA analysis.

Multidimensional Gas Chromatography–Mass Spectrometry–Olfactometry (MD-GC-MS-O). MD-GC-MS-O was conducted using a selectable 1D/2D GC-MS system with an Agilent 7890A GC fitted with a low thermal mass (LTM) system.[12] The GC was coupled with a Gerstel cooled injection system CIS-4 and a FID (250ºC), a 5975 mass selective detector, and a sniffing port fitted with a Gerstel multi-column switching system. The effluent from the first column (DB-WAX capillary column; 0.25 mm i.d. × 30 m; film thickness 0.25 μm, LTM; Agilent

Technologies, Inc.) at the end of the capillary was divided into three branches and routed via deactivated fused silica capillaries to the FID, mass detector, and sniffing port. The branch routing could be switched with the column switching device to eliminate the compounds eluted from the first column or transfer them directly to the second column (DB-1 capillary column; 0.25 mm i.d. × 30 m; film thickness 0.25 μm; Agilent Technologies, Inc.). The effluent from the second column at the end of the capillary was also divided into three branches and routed via deactivated fused silica capillaries to the FID, mass detector, and sniffing port. Each sample (the concentrate of DHS sampling) was injected in 1 μL volumes in splitless mode. The injection temperature was maintained at 10°C for the first 0.5 min then increased to 240°C at 12°C/s. The oven temperature of the first column was kept at 40ºC then increased to 230ºC at 10ºC/min, maintaining a constant pressure of 362 kPa. The effluent was eliminated again when the target retention time (rotundone: 20.2–20.6 min, MPF: 21.8–22.6 min) was reached, whereupon the trapped material was heated to 250ºC and directed to the second column. The oven temperature of the second column was maintained at 40ºC for the initial time (rotundone: 21.0 min, MPF: 23.0 min), and then increased to 230ºC at 20ºC/min, maintaining a constant pressure of 362 kPa.

High-Resolution Mass Spectrometry (HRMS). HRMS was performed on an AccuTOF GCv 4G instrument (JEOL Ltd., Tokyo, Japan) fitted with an InertCap WAX capillary column (0.25 mm i.d. × 60 m, 0.25 μm film thickness; GL Sciences Co.). The injection conditions, oven temperature program, carrier gas, and flow rate were the same as those described above for GC-MS/FID.

Nuclear Magnetic Resonance (NMR) Spectroscopy. The ^{1}H NMR (400 MHz, $CDCl_3$, residual $CHCl_3$ at $\delta = 7.26$ as internal standard) and ^{13}C NMR (100 MHz, $CDCl_3$, $CDCl_3$ at $\delta =$ 77.0 as internal standard) experiments were conducted using a JNM-ECX 400 spectrometer

(JEOL Ltd., Tokyo, Japan). The chemical shifts and coupling constants (*J*) are expressed in parts per million (ppm) and hertz (Hz), respectively.

Odor Threshold Definition of MPF. The odor threshold of MPF in water was evaluated using a previously reported method.[13] Panelists [n = 23 (16 males and 7 females; age 20–60 years)] were employees of the R&D Center of T. Hasegawa Co., Ltd. and were trained to recognize and quantify aromas using approximately 100 odorous chemicals and raw materials. The MPF concentrations used were 1.3, 6.4, 32, 160, 800, 4,000, 20,000, and 100,000 ng/kg. The most concentrated samples were prepared by diluting an ethanol solution of MPF 10,000 times with water. Equal volumes of ethanol were added to the corresponding blank samples. Two grams of each sample were added to a closed sensory vial (total volume 30 mL) and allocated a random three-digit numerical code. The samples were presented in ascending order of sample concentration. Three samples in a series were presented in random order and identified only by their three-digit random numbers. The spiked sample always differed from the other two. Orthonasal assessments were performed in a quiet room maintained at 23°C. Along with the threshold assessment, panelists assessed the odor of the sample they had successfully recognized. All panelists were assigned a best estimate threshold value, which was the geometric mean of the highest concentration missed and next highest concentration tested. The geometric mean of the individual best estimate threshold was then calculated to afford the threshold value.

Triangle Test. The triangle test was conducted to evaluate the effects of rotundone and MPF on the rose aroma reconstitutes of *R. damascena*. The panel comprised 52 panelists (30 males and 22 females; age 20–60 years), who were employees of the R&D Center of T. Hasegawa Co., Ltd. and were trained to recognize and rate intensities using approximately 100 odorous chemicals and raw materials. Four aroma reconstitutes (samples A–D) were assessed. The aroma

reconstitutes were produced by mixing dipropylene glycol with the odorants showing a high FD factor by AEDA at concentrations based on the GC peak area percentages of the DHS analysis described previously. Aroma reconstitute A comprised seven compounds [2-phenylethanol (20 g/kg), citronellol (12 g/kg), geraniol (10 g/kg), nerol (7.0 g/kg), citral (mixture of neral and geranial; 0.75 g/kg), rose oxide (0.13 g/kg), and linalool (0.075 g/kg)]. Aroma reconstitutes B, C, and D were produced by adding rotundone (50 μg/kg), MPF (5 μg/kg), and rotundone (50 μg/kg) and MPF (5 μg/kg) to aroma reconstitute A, respectively. A 2 g portion of each sample was placed in a closed sensory vial (total volume 10 mL) and allocated a random three-digit number as a code. Each panelist was presented with a series of test samples (AAB, ABB, AAC, ACC, AAD, or ADD) with instructions to sniff each sample and identify samples B, C, or D against A. Simultaneously, the panelists assessed the odor of the sample they had successfully recognized.

Identification of Rotundone and MPF in Various Types of Roses. The headspace gases of the living flowers of *R. centifolia* 'Bullata,' *R.* 'Neige Parfum,' *R.* 'Pope John Paul II,' *R.* 'Lady Hilingdon,' and *R.* 'Grand Mogul' were pumped through 0.4 g of Tenax TA (0.5 L/min, 3 h) at 22°C–25°C. The adsorbents were eluted with 4 mL each of pentane and diethyl ether. The eluents were concentrated and subjected to MD-GC-MS-O to tentatively identify rotundone and MPF using the procedure described above.

RESULTS AND DISCUSSION

DHS Analysis and AEDA. DHS sampling was used to prepare the headspace aroma concentrate of *R. damascena* 'trigintipetala.' If the flowers are left unpicked until daytime, their scent weakens and deteriorates. Thus, the flower petals were hand-picked in the morning. When using only the petals, the flower scent remained constant during a six-hour sampling. Moreover,

sampling from the petals of 20 flowers reduced the influence of individual differences between flowers. The olfactory properties of the aroma concentrate represented those of the living flower. We conducted AEDA to define odor-active compounds in the aroma concentrate of *R. damascena*. Consequently, along with major compounds including 2-phenylethanol, geraniol, citronellol, and nerol, two ultra-trace components (unknowns A and B) with a high FD factor (FD 625) were detected as odor-active compounds (**Table 1**). These results were different from those of the reported rose oil[7]; rose oxide, *trans*-nerolidol, and geranyl butyrate showed the highest FD factor in AEDA analysis, whereas the compounds corresponding to unknown A and B were not detected.

Elucidation of Unknown Compounds. Unknown A was identified as rotundone, with a woody note, by MD-GC-MS-O analysis using the same retention time, MS, and comparing the odor qualities with those of the authentic synthesized rotundone (**Figure 2**). Rotundone was identified as an odor-active component in patchouli oil,[14] frankincense oil,[15] Shiraz wine,[16] peppers,[16] and several fruits (grapefruit, orange, apple, and mango).[9] To the best of our knowledge, rotundone has not been reported in roses.

However, a clear mass spectrum of unknown B (citrus note) was obtained by MD-GC-MS-O, although its structure could not be identified by MS library matching. Therefore, we attempted to obtain sufficient quantities of the compound for NMR experiments. The target citrus-like compound was detected in commercial rose absolute from *R. damascena*. Thus, it was isolated from rose absolute by several fractionation steps. First, molecular distillation was performed to remove nonvolatile components from the absolute. Then, fractional distillation was conducted to separate the target compound from 2-phenethyl alcohol, which is a major constituent (approximately 60%) of the volatile fraction. Subsequent fractionation via silica gel column

chromatography and two-step preparative HPLC successfully purified the target compound. As confirmed by GC-FID, the final purity of the isolated compound (2.6 mg) was 97%. This was assumed to be 4-(4-methylpent-3-en-1-yl)-2(5*H*)-furanone (MPF; **1**) from high-resolution mass and NMR spectra. Identification was verified further by matching the analytical data and odor qualities of the isolated compound with those of the synthesized MPF (**Figure 3**). In a preamble to a report on rose oil analysis by Kovats,[6] Ohloff and Demole reported MPF in rose oil.[5] However, details (quantity, character, and odor) of MPF were unmentioned. MPF was also identified in the secretions of acarid mites and tentatively named as *α*,*β*-acariolide,[17] but its biological function remains unclear. Although headspace analysis of the flower of several types of hybrid rose cultivars were reported[18, 19, 20], rotundone and MPF were not identified. To the best of our knowledge, this is the first study that reports the organoleptic properties of MPF. In the threshold measurement of MPF, panelists reported that this emitted a citrus-like (lemon, orange, and grapefruit) and floral odor (muguet and jasmine) with a moderately low threshold of 3.6 μg/kg in water.

Evaluation of the Effects of Rotundone and MPF. A triangle test was conducted to investigate the effects of rotundone and MPF on rose aroma reconstitutes of *R. damascena*. The concentrations of rotundone and MPF were initially settled at levels where the characteristic odor (the woody odor of rotundone and the citrus-like odor of MPF) was not sensorially distinctly recognized. **Figure 4**[21] shows the results of this test. Aroma reconstitutes B and C were not significantly discriminated from aroma reconstitute A. Only aroma reconstitute D was significantly discriminated from aroma reconstitute A. Furthermore, panelists who could discriminate between aroma reconstitutes D and A assessed the aroma of D as “more blooming than A” or “more natural than A.” The panelists did not distinguish aroma reconstitutes A and D

as "woody" or "citrus-like." The effects of these two added compounds are expected to differentiate the natural rose aroma from that of artificial rose aroma reconstitutes.

Identification of Rotundone and MPF in Various Types of Roses. A further question was whether these two compounds existed in other roses. To verify this, MD-GC-MS-O analysis was used to investigate the existence of rotundone and MPF in five types of fragrant roses. *R. centifolia* is one of the two main species with *R. damascena* cultivated for the perfume industry[1]. *R. centifolia* 'Bullata' is one of the cultivars of *R. centifolia*. Other four types of roses are the hybrid tea rose cultivars that emit a strong and rich fragrance. Rotundone was detected in all five types, whereas MPF was detected in three types (i.e., *R. centifolia* 'Bullata,' *R.* 'Neige Parfum,' and *R.* 'Pope John Paul II'). These results indicate that rotundone and MPF are widely distributed not only in *R. damascena* but also in several types of rose cultivars and contribute to their beautiful aroma.

In conclusion, this is the first study to identify rotundone and MPF as potent aroma compounds that are emitted from the blooming flower of *R. damascena.* The addition of rotundone and MPF to the aroma reconstitute of *R. damascena* provided it with blooming and natural characteristics. Furthermore, rotundone and MPF were tentatively identified by matching the RIs and odor qualities in several fragrant roses, including the hybrid tea rose cultivars. To confirm the contributions of rotundone and MPF to the aromas of the natural rose flowers, quantitative studies must be conducted with respect to the headspace volatile composition of the flowers.

ABBREVIATIONS USED

DHS, dynamic headspace; AEDA, aroma extract dilution analysis; GC-O, gas chromatography–olfactometry; GC-MS-FID, gas chromatography–mass spectrometry–flame ionization detection; EI, electron impact; FID, flame ionization detector; RI, retention indices; MD-GC-MS-O, multidimensional gas chromatography–mass spectrometry–olfactometry; NMR, nuclear magnetic resonance; HRMS, high-resolution mass spectra; HPLC, high-performance liquid chromatography; MPF, 4-(4-methylpent-3-en-1-yl)-2(5*H*)-furanone.

ETHICS & CONFLICT OF INTEREST

The authors declare no competing financial interest.

REFERENCES

(1) Baldovini, N.; Filippi, J.-J. Natural Fragrant Raw Materials. In *Springer Handbook of Odor*; 1st ed.; Springer: Cham, 2017; pp 46−48.

(2) Lawrence, B. Progress in Essential Oils. *Perfum*. *Flavor*. **1991**, *16*(*3*), 43–77.

(3) Lawrence, B. Progress in Essential Oils. *Perfum*. *Flavor*. **2005**, *30*(*4*), 60–74.

(4) Seidel, C. F.; Stoll, M. Investigation of rose oil. I. Low boiling constituents of Bulgarian rose oil. *Helv*. *Chim*. *Acta*. **1959**, *42*, 1830–1844.

(5) Ohloff, G.; Demole, E. Importance of the odoriferous principle of Bulgarian rose oil in flavour and fragrance chemistry. *J*. *Chromatog*. **1987**, *406*, 181–183.

(6) Kovats, E. S. Composition of essensial oils: Part 7. Bulgarian oil of rose (*Rosa damascena* Mill.). *J*. *Chromatog*. **1987**, *406*, 185–222.

304 (7) Xiao, Z.; Li, J.; Niu, Y.; Liu, Q.; Liu, J. Verification of key odorants in rose oil by gas
305 chromatography−olfactometry/aroma extract dilution analysis, odour activity value and
306 aroma recombination. *Nat*. *Prod*. *Res*. **2017**, *31*, 2294–2302.

307 (8) Rusanov, K.; Kovacheva, N.; Stefanova, K.; Atanassov, A.; Atanassov, I. *Rosa damascena*–
308 genetic resources and capacity building for molecular breeding. *Biotechnol*. *& Biottechnol*.
309 *Eq*. **2009**, *23*(*4*), 1436–1439.

310 (9) Nakanishi, A.; Fukushima, Y.; Miyazawa, N.; Yoshikawa, K.; Maeda, T.; Kurobayashi, Y.
311 Identification of rotundone as a potent odor-active compound of several kinds of fruits. *J*.
312 *Agric*. *Food Chem*. **2017**, *65*, 4464–4471.

313 (10) Poulter, C. D.; Wiggins, P. L.; Plummer, T. L. Synthesis of fluorinated analogs of
314 geraniol. *J. Org. Chem.* **1981**, *46*, 1532–1538.

315 (11) Grosch, W. Determination of potent odourants in food by aroma extract dilution analysis
316 (AEDA) and calculation of odour activity values (OAVs). *Flavour*. *Frag*. *J*. **1994**, *9*, 147–
317 158.

318 (12) Sasamoto, K.; Ochiai, N. Selectable one-dimensional or two-dimensional gas
319 chromatography-mass spectrometry with simultaneous olfactometry or element-specific
320 detection. *J*. *Chromatogr*. *A*. **2010**, *1217*, 2903–2910.

321 (13) Meilgaard, M.; Civille, G. V.; Carr, B. T. *Sensory Evaluation Techniques*, 5th ed.; CRC
322 Press: Boca Raton, FL, 2016; pp 153−163.

(14) Kaiser, R. Agarwood–the wood of the God. In *Meaningful Scents around the World*. *Olfactory, Chemical, Biological and Cultural Considerations*; Kisakürek, M. V., Eds.; Verlag Helvetica Chimica Acta: Zürich, Wiley-VCH: Weinheim, 2006; pp 59–64.

(15) Niebler, J.; Zhuravlova, K.; Minceva, M.; Buettner, A. Fragrant sesquiterpene ketones as trace constituents in frankincense volatile oil of *Boswellia sacra*. *J. Nat. Prod.* **2016**, *79*, 1160–1164.

(16) 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.; Herderich, M. J. From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. *J. Agric. Food Chem.* **2008**, *56*, 3738–3744.

(17) Tarui, H.; Mori, N.; Nishida, R.; Okabe, K.; Kuwahara, Y. 3-(4-Methyl-3-pentenyl)-2(5*H*)-furanone, *α*,*α*-Acariolide and 4-(4-Methyl-3-pentenyl)-2(5*H*)-furanone, *α*,*β*-Acariolide. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 135–140.

(18) Brunke, E.-J.; Hammerschmidt F.-J.; Schmaus G. Scent of roses−recent results. *Flavour Frag. J.* **1992**, *7*, 195–198.

(19) Bianchi, G.; Nuzzi, M.; Leva, A. A.; Rizzolo, A. Development of a headspace-solid phase micro extraction method to monitor changes in volatile profile of rose (*Rosa hybrida*, cv David Austin) petals during processing. *J. Chromatogr. A*. **2007**, *1150*, 190–197.

(20) Joichi, A.; Nakamura, Y.; Haze, S.; Ishikawa, T.; Atoji, H.; Nishida, T. Sakurai, K. Volatile constituents of blue-coloured hybrid tea rose flowers. *Flavour Frag. J.* **2013**, *28*, 180–187.

(21) Ohashi, T.; Miyazawa, Y.; Shibuya, T.; Ishizaki, S.; Kurobayashi, Y.; Saito, T. Identificatoin of odor-active trace compounds in Damask rose (*Rosa damascena*). In *Flavour Science: Proceedings of the XV Weurman Flavour Research Symposium*, B. Siegmund, & E. Leitner (Eds.); Verlag der Technische Universität Graz., 2018; pp 47−50.

Figure Captions

Figure 1. Synthesis of 4-(4-methylpent-3-en-1-yl)-2(5*H*)-furanone.

Figure 2. Overlay of the total ion chromatograms of rotundone reference material (in blue) and *Rosa damascena* aroma concentrate (in black) with the mass spectra of each.

Figure 3. Comparison of mass spectra and ^{1}H NMR spectra of the isolated target citrus-like compound and those of authentic MPF.

Figure 4. Accuracy rate of the triangle test (Identification of sample B, C, or D against A). A: control (-rotundone, -MPF), B: +rotundone (50 µg/kg), C: +MPF (5 µg/kg), D: +rotundone (50 µg/kg), +MPF (5 µg/kg) n = 52, *Binominal test, $p < 0.05$

Table 1. Odor-active compounds (FD ≥ 25) in the *Rosa damascena* aroma concentrate

no.	odorant	odor quality[a]	RI on InertCap WAX	FD factor	GC peak area, %[b]	identification mode[c]
1	(*Z*)-rose oxide	rose, green	1361	3125	0.21	MS, RI, GC-O
2	linalool	floral, fresh	1549	125	0.06	MS, RI, GC-O
3	geranial	rose, fresh	1752	25	0.87	MS, RI, GC-O
4	citronellol	rose, fresh	1770	3125	20.38	MS, RI, GC-O
5	nerol	rose, fresh	1810	25	11.83	MS, RI, GC-O
6	geraniol	rose, fresh	1853	3125	15.80	MS, RI, GC-O
7	2-phenylethanol	rose, honey	1930	15625	32.64	MS, RI, GC-O
8	eugenol	clove-like	2188	3125	trace	MS, RI, GC-O
9	γ-undecalactone	lactone	2288	625	trace	RI, GC-O
10	unknown A	woody	2300	625	trace	
11	unknown B	citrus-like	2466	625	trace	

[a] Odor quality perceived at the sniffing port. [b] trace, trace amount (<0.001%).

[c] MS, reference mass spectrum; RI, retention indices; GC-O, gas chromatography–olfactometry.

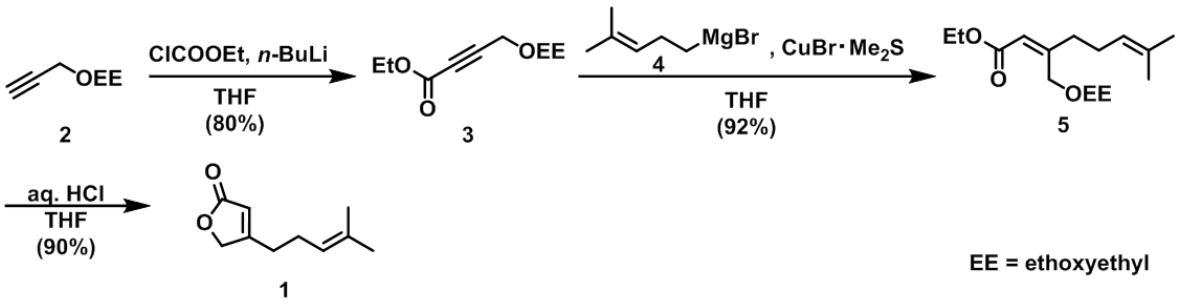
OEE
2
ClCOOEt, n-BuLi
THF
(80%)
EtO
O
OEE
3
MgBr
4
, CuBr·Me2S
THF
(92%)
EtO
O
OEE
5
aq. HCl
THF
(90%)
O
O
1
EE = ethoxyethyl

Figure 1.

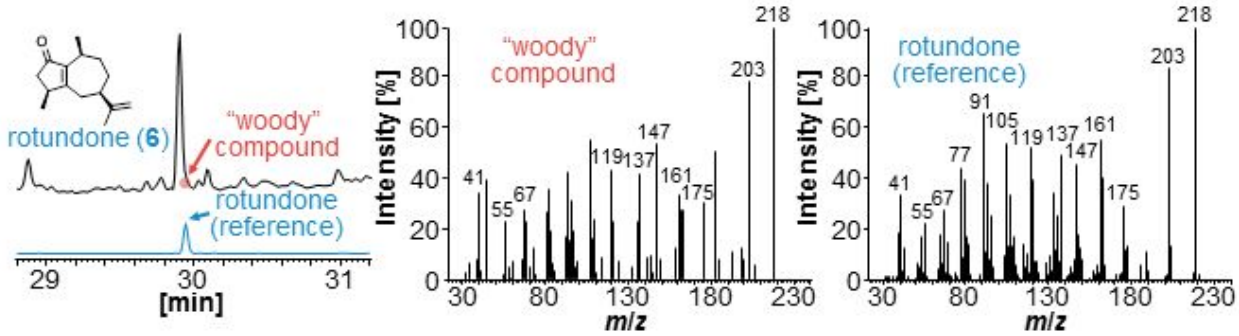
rotundone (6)
"woody" compound
rotundone (reference)
29
30
31
[min]
"woody" compound
Intensity [%]
m/z
rotundone (reference)
Intensity [%]
m/z

Figure 2.

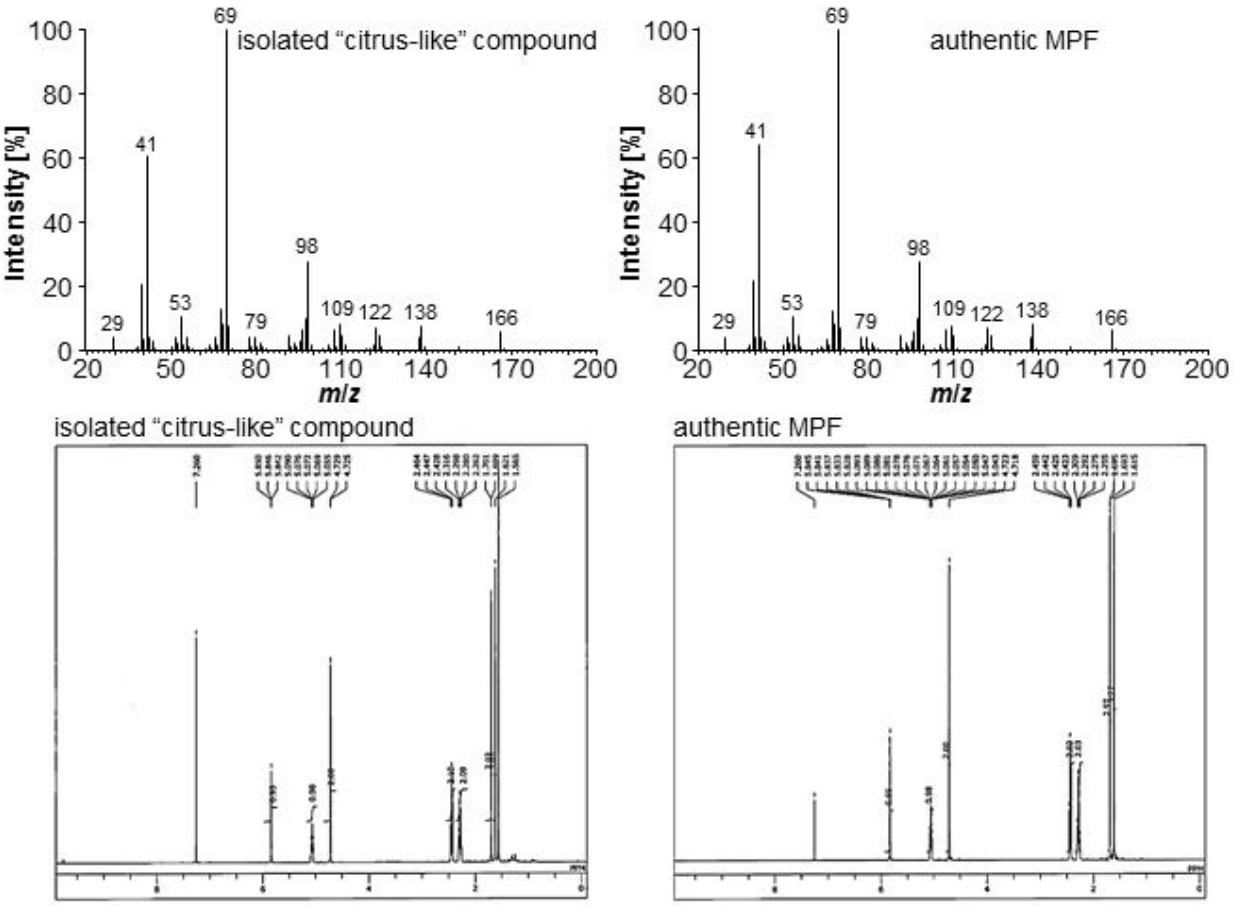
isolated "citrus-like" compound
authentic MPF
Intensity [%]
m/z
69
41
98
53
29
79
109
122
138
166
isolated "citrus-like" compound
authentic MPF

Figure 3.

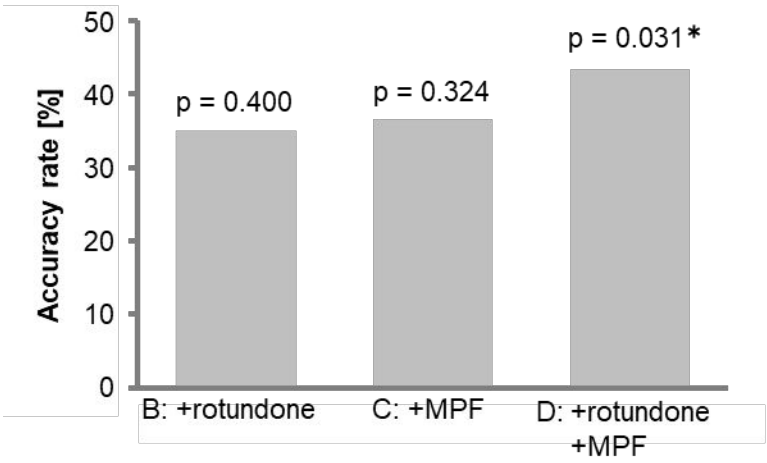
Accuracy rate [%]
50
40
30
20
10
0
p = 0.400
p = 0.324
p = 0.031*
B: +rotundone
C: +MPF
D: +rotundone +MPF

Figure 4.

TOC/Abstract Graphic