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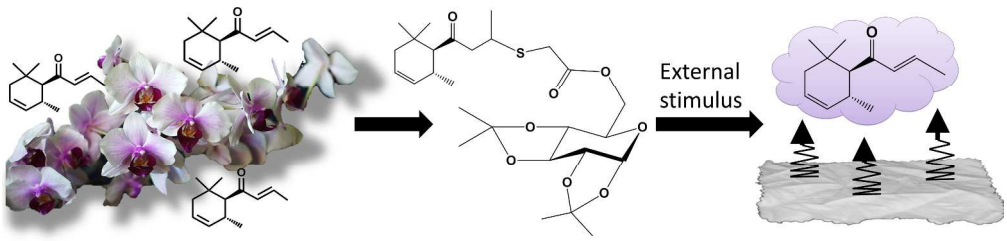

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Controlled fragrance release from galactose-based pro-fragrances

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ABSTRACT

The volatile nature of olfactory compounds has led to the development of pro-fragrances, which slowly release the active fragrance molecules upon cleavage of a chemical bond to a substrate. Based on the hypothesis that monosaccharide motifs could serve to effectively anchor pro-fragrances on cotton, which is an important requirement for use in laundry products, we investigated new galactose-based pro-fragrances. A retro 1,4-Michael-type reaction was employed as the release mechanism. Thus, δ-damascone was reacted in a 1,4-addition with mercaptoacetic acid, and the product was coupled with 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose. To explore the influence of the molecules' polarity on the deposition and release kinetics, both the isopropylidene-protected hydrophobic as well as the deprotected hydrophilic pro-fragrance were studied. The fragrance release was investigated in aqueous solution by ^{1}H-NMR spectroscopy as a function of pH; the data show that both pro-fragrances are stable under acidic conditions, but release the δ-damascone under basic conditions. The release kinetics are well described by a first-order process, and observed to be much faster in case of the isopropylidene-protected hydrophobic pro-fragrance. The fragrance release from washed and dried cotton tissue was investigated via dynamic headspace analysis followed by gas chromatography – mass spectroscopy. The data show that the deposition from solution is much better for the hydrophobic pro-fragrance, that the δ-damascone is slowly released in both cases, and that the amount of δ-damascone that can be released is increased by over two orders of magnitude higher than in the case of tissue washed with the neat fragrance under identical conditions.

INTRODUCTION

The pleasant smell of plants, which employ volatile olfactory compounds as signaling elements, has led to the use of natural extracts as fragrances or perfumes already thousands of years ago.[1-3] Over the last century the industrial importance of fragrances has evolved from the traditional use in perfumes, personal care products, cleaning and laundry products to many other consumer products. In many applications the products are expected to provide a characteristic scent over a long time, but this objective appears to be at odds with the volatile nature of olfactory compounds, which is of course needed to permit efficient evaporation and transport. In Nature several mechanisms have evolved to solve this dilemma. Especially plants use different kinds of "precursors", such as fatty or amino acids, carotenoides,[4] and glycosides[5] to store, carry, and release volatile olfactory compounds. Mimicking this general approach, a broad range of physical and chemical mechanisms to release highly volatile compounds over extended periods of time have been developed. Physical release systems typically rely on the encapsulation of fragrances, for example by enclosing them with a polymer, either in the form of a membrane through which they can diffuse slowly, or a hard capsule, from which they are released upon breakage of the shell.[6-9] In chemical release systems the fragrance molecules are usually covalently attached to a substrate, which can be another small molecule, a macromolecule, a nanoparticle, or any other suitable substrate.[10-12] Possible advantages of covalent bonds over other interactions are a higher stability of the delivery system in different consumer formulations as well as the possibility to selectively influence the performance of the pro-fragrance by modulating the release kinetics, the polarity, or other parameters. A careful evaluation of performance to cost ratio has to be done for each particular application. The covalent attachment of the fragrance molecules to substrates results in so-called pro-fragrances,[10] i.e., non-volatile and odorless precursor molecules from which the active olfactory agents can be released upon selective cleavage of the covalent bond that connects the fragrance with the carrier. Ideally the release can be triggered by a specific stimulus, i.e., exposure to heat, light, a change in pH, etc. Many different chemical reactions have been studied in this context.[10,13-21] Polymers are often used as a scaffold for pro-fragrances, as these show generally slower release kinetics than low-molecular weight counterparts and are able to sustain the fragrance release for extended periods of time.[10,13,15-21] General drawbacks of polymers are, however, the significant mass added by the scaffold (resulting in a small payload), and their often very limited biodegradability.[22] Polysaccharides, such as cyclodextrins, have also been studied as systems for fragrance delivery.[23] Nevertheless, these release systems are

based on host-guest interactions, which are limited in their application due to substrate specificity or limited capacity for fragrance storage.[23]

Based on the hypothesis that monosaccharide motifs could serve to effectively anchor pro-fragrances on cotton, which is an important requirement for use in laundry products,[15] we embarked on the investigation of new galactose-based pro-fragrances. Besides their possible interactions with cotton through hydrogen bonding, saccharides offer many other attractive features, including their renewable nature, abundance, low cost, variety, biodegradability, and water-solubility. The free hydroxyl groups can serve to covalently attach the payload, as well as auxiliary chemical motifs which may serve as anchors, solubilizing groups, or to change the polarity of the substrate from hydrophilic to hydrophobic. As it has been shown that the polarity of the structure can significantly influence the deposition of a pro-fragrance onto a substrate and also affect the release kinetics,[13,24] this latter feature appears to be particularly important for practical applications. The polarity of fragrance molecules can be expressed by the log $P_{o/w}$ which is the logarithmic octanol/water partition coefficient,[25,26] which can be calculated from the chemical structure.

Interestingly, examples of monosaccharide-based pro-fragrances are rare. Most activities in this domain have centered around glycosidically-bonded volatiles.[27,28] These precursors are widely found in Nature and release their fragrances upon enzymatic cleavage or digestion by microorganisms.[5] This mechanism has been used for insect repellents and bodycare products, where glycosidases from the skin release the payload in the form of an alcohol.[28-30] As the glycosidic bond can also be cleaved at temperatures above 200 °C, glycosidic precursors were also tested in cigarettes.[31] The release mechanism exploited here, however, did not involve the glycosidic bond. Instead, we opted to employ a retro 1,4-Michael-type reaction, which has been widely used to release α,β-unsaturated ketones such as damascones and ionones[13,14,16,21,32] and represents a broadly useful release scheme. As the addition of thiols to α,β-unsaturated ketones has been reported to be more efficient than alcohols,[32] we chose to employ an alkyl thiol linker between the primary hydroxyl group of galactose. We used (±)-*trans*-δ-damascone [(±)-(*E*)-1-((1*RS*,2*SR*)-2,6,6-trimethylcyclohex-3-en-1-yl)but-2-en-1-one] as a typical representative of the damascone family. Both a hydrophobic and a hydrophilic pro-fragrance were made to study the slow release of fragrances molecules after 3 days of drying as well as the effect of the precursor's polarity on the deposition onto cotton and the release kinetics under various hydrolytic conditions.

EXPERIMENTAL

Materials and general methods

Commercially available reagents and solvents were used without further purification, unless otherwise mentioned. Reactions were carried out in standard glassware under N_2. ^1H and ^{13}C NMR spectra were acquired on a Bruker Avance III 300 MHz spectrometer and chemical shifts are reported in ppm relative to internal solvent peak as a standard. Infrared spectra were recorded on a Perkin Elmer Spectrum 65 spectrometer in ATR mode; peak positions are expressed in cm^{-1} and the absorption at each peak was qualified as weak (w), medium (m), strong (s). Mass spectra were recorded on a Bruker Esquire HTC mass spectrometer. The log $P_{o/w}$ values were calculated using the *EPI Suite PBT Calculator 1.0.0* based on the *EPIwin* program, *US Environmental Protection Agency* 2000.

1,2:3,4-Di-O-isopropylidene-α-D-galactopyranose

1,2:3,4-Di-O-isopropylidene-α-D-galactopyranose was prepared using the procedure reported by Gille and Hiersemann.[33]

(±)-2-((4-Oxo-4-((1*SR*,2*RS*)-2,6,6-trimethylcyclohex-3-en-1-yl)butan-2-yl)thio)acetic acid (1)

(±)-(*E*)-1-((1*RS*,2*SR*)-2,6,6-Trimethylcyclohex-3-enyl)-but-2-en-1-one (δ-damascone, 5.0 g, 26 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.4 mL, 2.6 mmol) were dissolved in methanol (10 mL) before thioglycolic acid (2.3 mL, 31 mmol) was added and the solution was stirred at RT for 24 h. Thin layer chromatography showed complete conversion at this time. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (10 mL) and washed consecutively with 0.5 M HCl (5 mL), water (5 mL) and brine (5 mL). The crude product was purified by flash chromatography using hexane and ethyl acetate (1:3) as eluent, to afford the title compound as a yellow viscous oil (in a 1:1 commercially available diastereoisomeric mixture, 7.25 g, 98%). ^1H NMR (300 MHz, $CDCl_3$): δ = 5.53 (m, 1H), 5.43 (m, 1H), 3.42 (m, 1H), 3.35 (m, 2H), 3.00-2.52 (m, 2H), 2.50 (m, 1H), 2.21 (dd, 10.6; 3.1Hz, 1H), 1.96 (m, 1H), 1.69 (m, 1H), 1.34 (m, 3H), 0.97 (m, 3H), 0.95 (m, 3H), 0.9-0.86 (m, 3H) ppm. ^{13}C NMR: (100.6 MHz, $CDCl_3$): δ = 212.43 (s), 212.23 (s), 175.82 (d), 131.77 (s), 131.66 (s), 124.28 (s), 124.12 (s), 62.84 (d), 54.76 (s), 54.61 (s), 41.68 (d), 35.58 (d), 33.17 (d), 33.09 (s), 32.91 (s), 31.77 (s), 31.65 (s), 29.76 (d), 21.33 (s), 21.14 (s), 20.71 (s), 19.88 (s) ppm. IR (neat): 3019w, 2958m, 2872m, 1704s, 1458w, 1366m, 1296m, 1116m, 1009m, 933w, 896w, 689s, 640w cm^{-1}. MS: m/z calcd. for $C_{15}H_{24}O_3S$, $[M + Na]^+$ 307.1338, found 307.1342. 62.5% of the total weight is related to the neat fragrance.

((3a*R*,5*R*,5a*R*,8a*S*,8b*R*)-2,2,7,7-Tetramethyltetrahydro-3a*H*-bis([1,3]dioxolo)[4,5-b:4',5'-d]pyran-5-yl)methyl 2-((4-oxo-4-((1*SR*,2*RS*)-2,6,6-trimethylcyclohex-3-en-1-yl)butan-2-yl)thio)acetate (2)

Compound **1** (0.7 g, 2.5 mmol) and 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (0.7 g, 2.7 mmol)

were dissolved in dichloromethane (DCM, 10 mL), 1,3-dicyclohexylcarbodiimide (DCC, 0.6 g, 3.0 95 mmol) and 4-(dimethylamino)-pyridine (DMAP, 0.1 g, 0.2 mmol) were added and the reaction mixture was stirred at RT for 1 h. The white precipitate that had formed during the reaction was filtered off and the filtrate was washed with water (15 mL) and brine (15 mL). The organic layer was concentrated under reduced pressure and the crude product was purified by flash column chromatography using hexane and ethyl acetate (9:1; v/v) as eluent, to afford the title compound as a yellow viscous oil (0.92 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.55-5.50 (m, 2H), 5.45 (m, 1H), 4.61 (dd, 7.9, 2.5 Hz, 1H), 4.37-4.30 (m, 2H), 4.26-4.19 (m, 2H), 4.04 (m 1H), 3.42 (m, 1H), 3.38-3.26 (m, 2H), 3.00-2.44 (m, 3H), 2.20 (m, 1H), 1.96 (m, 1H), 1.69 (m, 1H), 1,51 (s, 3H), 1,44 (s, 3H), 1,33-1.30 (m, 9H), 0.98-0.93 (m, 6H), 0.89-0.86 (m, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 212.10 (m), 175.02 (d), 131.80 (d), 124.17 (d), 109.65, 108.79, 96.26, 71.00 (d), 70.69, 70.49, 65.80 (d), 64.22 (m), 62.82 (d), 62.72 (d), 41.72 (d), 35.40 (d), 33.11 (d), 32.85 (m), 31.62 (d), 29.75, 26.07, 25.95, 24.96, 24.49, 21.21 (m), 20.71, 19.88 (d) cm^{-1}. IR (neat): 2960m, 2933m, 1736m, 1706m, 1654w, 1456m, 1371m, 1254m, 1211m, 1166m, 1113m, 1068s, 1001s, 896m, 736m, 691m cm^{-1}. ESI MS: m/z calcd. for $C_{27}H_{42}O_8S$, $[M + Na]^+$ 549.2493, found 549.2490. 35.0% of the total weight is related to the neat fragrance.

((2*R*,3*S*,4*S*,5*R*,6*S*)-3,4,5,6-Tetrahydroxytetrahydro-2*H*-pyran-2-yl)methyl 2-((4-oxo-4-((1*SR*,2*RS*)-2,6,6 trimethylcyclohex-3-en-1-yl)butan-2-yl)thio)acetate (3)

Compound **2** (0.3 g, 0.6 mmol) was dissolved in a mixture of trifluoroacetic acid and water (5 mL, 9:1; v/v) and the solution was stirred for 30 min, before toluene (5 mL) was added and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography using chloroform and methanol as eluent (9:1; v/v) to afford the title compound in the form of pale yellow crystals (0.24 g, 94%). ^{1}H NMR (300 MHz, MeOH-D_4): δ = 5.57 (m, 1H), 5.47 (m, 1H), 5.16 (m, 0.5H Hα), 4.46 (m, 0.5H Hβ), 4.29 (m, 2H), 3.87 (m 1H), 3.78 (m, 1H), 3.49 (m, 1H), 3.41 (m, 1H), 3.36 (m, 2H), 3.07-2.67 (br, m, 2H). 2.47 (m, 1H), 2.33 (m, 1H), 2.03 (m, 1H), 1.73 (m, 1H), 1.32 (m, 4H), 1.01 (d, 7.2 Hz, 3H), 0.92 (m, 6H) ppm. ^{13}C NMR (75 MHz, CD_3OD): δ = 213.12, 170.95, 131.38 (d), 124.00 (d), 92.88, 73.40, 72.23, 69.57, 68.90, 62.37, 54.45, 41.29 (d), 34.87, 32.67 (d), 32.0, 31.60 (d), 28.76 (d), 22.82, 20.21 (br, d), 19.76, 18.84 (d) ppm. IR (neat): 3384m (br), 2960m, 2874m, 1733s, 1704s, 1456m, 1367m, 1279w, 1204w, 1136s, 1070s, 895w, 799m, 690m. MS: m/z calcd. for $C_{21}H_{34}O_8S$, $[M + Na]^+$ 469.1867, found 469.1863. 40.9% of the total weight is related to the neat fragrance.

Release of δ-damascone from pro-fragrances in aqueous buffer solutions

Aqueous buffer solutions of pH 4, 7, and 10 were prepared according to established protocols (see

supporting information)[34] using deuterium oxide instead of distilled water to facilitate NMR spectroscopy experiments. Pro-fragrances **2** and **3** (15 and 13 mg) were dissolved in deuterated methanol (MeOH-D_4, 1.5 mL) and just before starting the measurements the buffer solutions (100 µL) were added. The samples were kept at ambient temperature (20 - 25 °C) and the δ-damascone release was monitored by ^{1}H NMR spectroscopy in 30 min or 1 h intervals over the course of 24 h. The integrals of the appearing signals at 6.96 and 6.28 ppm, corresponding to the enolic double bond of the released δ-damascone, were compared to the integrals of the signal at 4.47 ppm, corresponding to proton (H-3) of the α-D-galactopyranose, and 2.50 ppm, corresponding to the unchanged proton (H-14) of the fragrance molecule.

Preparation of aqueous surfactant emulsions

A fabric softening surfactant emulsion was prepared as described previously[13,35] by combining 16.5% of the surfactant Stepantex® VK90, 0.2% of an aqueous calcium chloride solution (10%) and 83.3% water, pH ca. 3.1. An all-purpose surface cleaner (APC) formulation was prepared from Neodol® 91-8 (5.0%), Marlon® A 375 (4.0%), sodium cumolsulphonate (2.0%), Kathon® CG (0.2%) and water (88.8%). An aqueous solution of NaOH (50%) was added to adjust the pH to a value of 10.6. All % are given by weight.

Deposition of pro-fragrances and reference compounds onto ceramic tiles

Ceramic tiles were chosen for this test as they represent a natural surface that is widely used in buildings and therefore a good example for the contact with cleaning products. Pro-fragrances **2** and **3** (6.3, 5.3 mg), corresponding to a releasable amount of 2.4 mg of δ-damascone, were directly mixed under intense stirring with 1 mL of the APC detergent formulation until the emulsion appeared homogeneous to the unassisted eye. Before 9 mL of deionized water were added and the mixture was again well stirred for 1 min. 0.75 mL of this prepared solution were taken off and further put onto a pre-cleaned tile. All samples were prepared in duplicates respectively the neat reference. The tiles were further protected against dust and other environmental influences that could be easily deposit onto them and dried on the bench for 3 days.

Deposition of pro-fragrances and reference compounds onto cotton tissue

The deposition of pro-fragrances **2** and **3** onto cotton followed the protocol previously reported[13,35] (Figure 1). Compounds **2** and **3** were separately dissolved in ethanol (1 mL, **2**: 24 mg, **3**: 20 mg), corresponding to a releasable amount of 8.7 mg of δ-damascone. These solutions were separately added to the aqueous surfactant fabric softening emulsion (2 x 1.80 g) and the mixtures were stirred until the emulsions appeared to be homogeneous to the unassisted eye (ca. 5 min). The emulsions were

transferred to a 1 L beaker and diluted with deionized water (2 x 600 g). A cotton sheet (Swiss Federal Laboratories for Materials Science and Technology, cotton test cloth Nr. 221, cut into 12 x 12 cm sheets, average mass of 3.12 g, prewashed with an unperfumed detergent powder) was placed in each beaker and was manually stirred for 3 min, left to rest for 2 min, and manually wrung out (caution: use nitrile gloves for personal protection during the whole washing process) to obtain a wet cotton tissue with a total mass of around 7 g ± 0.1 g. This was repeated two times for each sample per series. The wet tissues were then line dried in a dark cupboard but otherwise ambient conditions for 3 days. Unmodified δ-damascone (8.7 mg) was deposited as reference in a similar manner.

We also directly deposited solutions of pro-fragrances **3** and **4** in organic solvents onto cotton tissue. Therefore pro-fragrance **3** and **4** (65, 70 mg) were dissolved in ethanol (2 x 500 µL) and 200 µL (containing a releasable amount of 8.7 mg of δ-damascone) of this solution was directly deposited onto the freshly washed cotton tissue. The cotton sheets were line dried in a dark cupboard for 3 days.

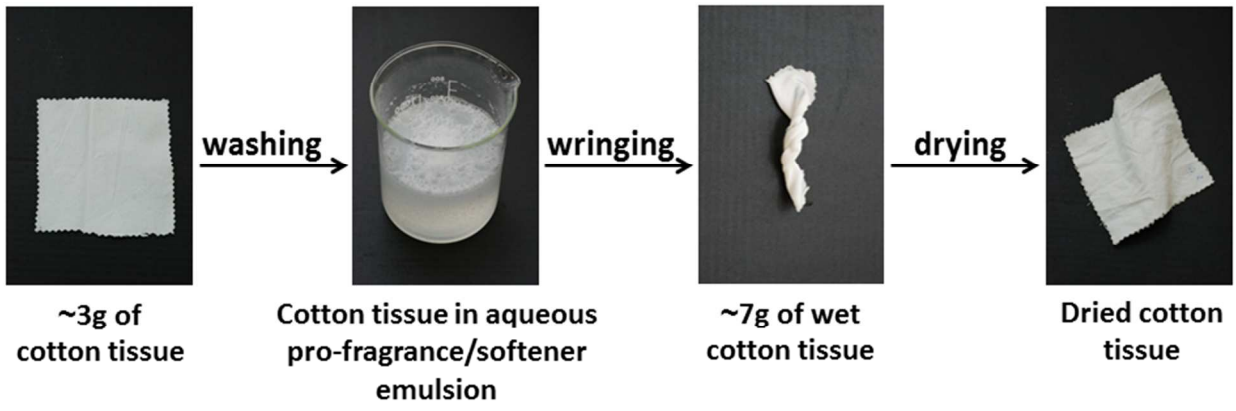


Figure 1. Pictures illustrating the deposition of pro-fragrances and the neat δ-damascone reference onto cotton tissue.

Dynamic headspace analysis of δ-damascone from treaded tiles and cotton tissue

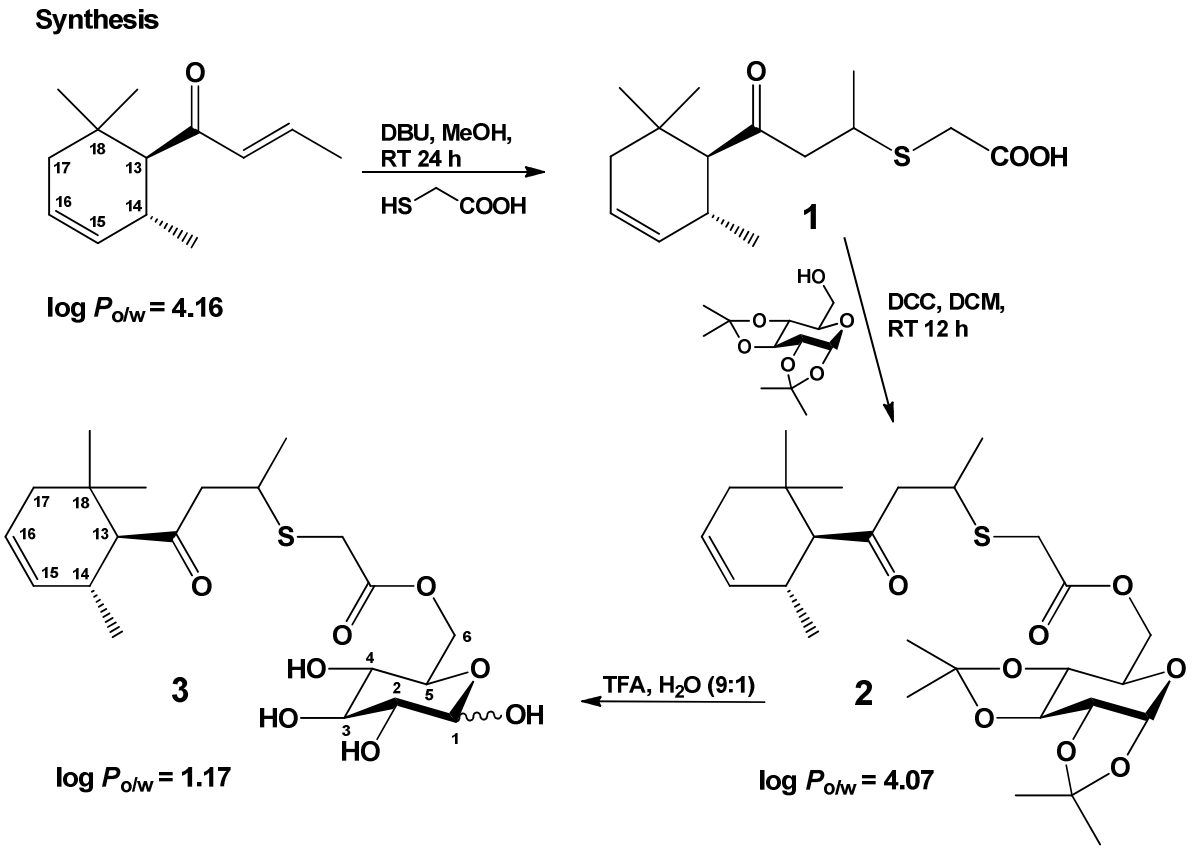
Dynamic headspace analysis was conducted according to the protocol previously reported.[35] The dried tiles and tissues, onto which either pro-fragrance **2** or **3** and the neat δ-damascone had been deposited, were placed into temperature-controlled (temperature = 25 °C) headspace sampling cells (volume = 160 mL) and exposed to a constant air flow (200 mL·min^{-1}). By passing the air first through active charcoal and a saturated NaCl solution a constant humidity of 75% was maintained. Tenax® cartridges (a commonly used absorber for organic volatiles) were used to adsorb the volatiles released from the tiles and the tissues. The system was first equilibrated for 15 min (using an old cartridge), before a fresh cartridge was inserted and the measurement was started. The collection lasted 15 min (corresponding to

a volume of 3 L of air) and repeated every 45 min for a period of 8 h. All measurements were carried out in duplicate. The sample cartridges were then thermally desorbed on a Perkin Elmer TurboMatrix ATD 350 desorber coupled to an Agilent Technologies 7890A gas chromatograph equipped with a HP-1 capillary column (30 m, i. d. 0.32 mm, film thickness 0.25 µm) and a flame ionization detector. The volatiles were analyzed using a two-step temperature gradient starting from 60 °C to 130 °C at 15 °C min^{-1} and then heating to 220 °C at 40 °C min^{-1}. The injection temperature was at 250 °C and the detector temperature at 250 °C. The amount of desorbed fragrance was determined via an external calibration curve using five different solutions of δ-damascone in ethanol. The obtained peak areas of δ-damascone in the GCMS-plots were plotted against the concentration of the injected solution.

RESULTS AND DISCUSSION

Synthesis of α-D-galactopyranose based pro-fragrances and model compounds

The targeted pro-fragrances **2** and **3** feature a thiol-ene adduct that connects the damascone to the primary alcohol group of α-D-galactopyranose and supports a retro 1,4-Michael-type reaction as the release mechanism (Scheme 1). Mercaptoacetic acid was chosen as it was the shortest commercially available mercapto acid, which reduces added weight due to the linker. We opted to first conduct the 1,4-addition of the linker with the δ-damascone, and couple the product with 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose, because thiols react selectively to double bonds. The linked fragrance serves then as a protecting group for the thiol to prevent it from oxidation or undesired linking to the galactose. In order to synthesize a well-defined pro-fragrance that carries only one fragrance molecule via a linker connected to the primary hydroxyl group, we utilized a protection scheme in which the secondary alcohol functions of the α-D-galactopyranose were protected through hydrophobic acetal groups. Since the latter can be cleaved without impacting the linker to the fragrance, this framework permitted the exploration of the deposition and the release characteristics of the hydrophobic intermediate **2** (log $P_{o/w}$ = 4.07) as well as the hydrophilic **3** (log $P_{o/w}$ = 1.17). As it has been shown for polymeric pro-fragrances that the release and the deposition efficiency onto cotton are determined *inter alia* by the hydrophilicity of the polymer carrier,[13] we deemed it desirable to explore differences between the two compounds at hand.



Scheme 1. Synthesis of pro-fragrances **2** and **3**. Intermediate **1** was prepared by the 1,4-addition of mercaptoacetic acid to δ-damascone. Esterification of **1** with protected 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose afforded pro-fragrance **2**, which was de-protected by hydrolysis to afford pro-fragrance **3**.

The first step of the synthesis of α-D-galactopyranose based pro-fragrances was the 1,4-addition of mercaptoacetic acid to a mixture of (1*R*,2*S*)- and (1*S*,2*R*)-isomers of (±)-(*E*)-*trans*-δ-damascone using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the catalyst. The resulting 1,4-addition product was isolated quantitatively and characterized to satisfaction by NMR spectroscopy and mass spectroscopy. In a second step, the adduct **1** was esterified with the protected galactose, using 1,3-dicyclohexylcarbodiimide (DCC) and catalytic amounts of 4-(dimethylamino)-pyridine (DMAP) to yield pro-fragrance **2** in good yield (Scheme 1). The ^{1}H NMR spectrum clearly shows the appearance of signals associated with the acetal protecting groups (four singlets at 1.51 -1.32 ppm) and resonances between 4.6 - 4.0 ppm which are diagnostic of the other protons of the galactose portion of the molecule. The remaining protons on the molecule are shifted only marginally during the attachment. Finally, the acetal groups of pro-fragrance **2** were removed by hydrolysis in a mixture of trifluoroacetic acid and water to afford pro-fragrance **3**, Figure 2 shows the IR spectra of the unbound fragrance and pro-fragrance **3**; this deprotection step is highly selective and neither influenced the ester nor the sulfide bond.

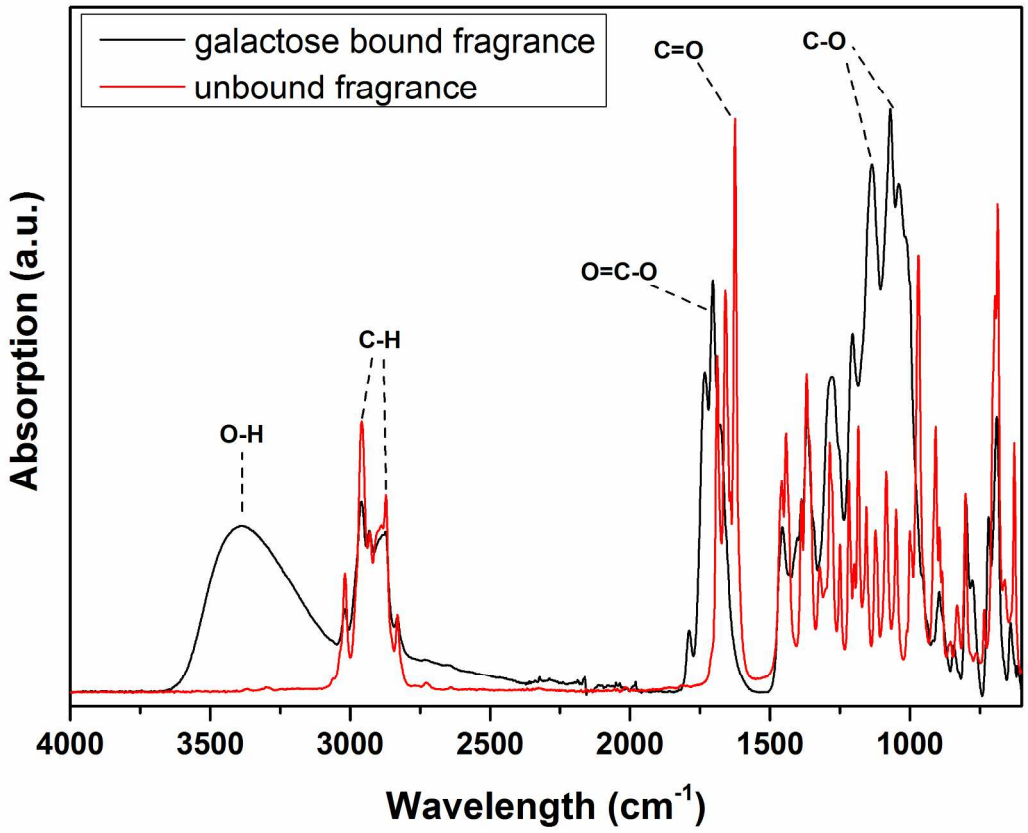


Figure 2. IR spectra of unbound fragrance and galactose bound pro-fragrance **3**.

Release of δ-damascone from pro-fragrances in aqueous buffer solutions at different pH

We first investigated the release kinetics of pro-fragrances **2** and **3** under hydrolytic conditions in buffered solutions at different pH (pH 4, pH 7 and pH 10) at ambient temperature (20 – 25 °C).[21] The experiments were conducted in a deuterated solvent so that ^{1}H NMR spectroscopy could be used to monitor the extent of released δ-damascone *in situ*. We note that the experiment was conducted in a closed system, which had an influence on the equilibrium between dissociation and formation reactions. Figure 3 shows as an example the ^{1}H NMR spectra acquired for the release from pro-fragrance **2** at pH 10 over the course of 24 h. The signals appearing at 6.96 and 6.28 ppm are diagnostic of the enonic double bond of the released δ-damascone and the ratio of the corresponding integrals to those of the unchanged signals at 4.47 ppm, corresponding to proton (H-3) of the α-D-galactopyranose, and 2.50 ppm, corresponding to the unchanged proton (H-14) of the fragrance molecule was plotted against time (Figure 4) to elucidate the release kinetics. A strong difference is seen between acidic conditions, under which both pro-fragrances **2** and **3** are stable over the time course investigated, and basic (and in case of **2** also neutral) conditions, under which the δ-damascone is released. This is consistent with the

mechanism for the release-step, which is initiated by deprotonation in the α-position to the ketone.[32] At pH 7, pro-fragrance **2** released the δ-damascone slowly and even after several days the equilibrium had not been reached. The release was much faster at pH 10 where an equilibrium concentration of the free fragrance was established after ca. 15 h. We note that during the NMR studies no degradation of the ester bond was detectable with our available methods. Figure 4 shows that in both cases the release is well described by:

$$A = A_0 + Ce^{-kt}$$

where A and A_0 represent the concentrations of the free fragrance at time t and time $t = 0$, respectively, and constants C and k are characteristic for the extent and rate of release. Based on the mathematical formula we assume that the release follows first order kinetics. In the case of pro-fragrance **2**, k takes values of -0.031 and -0.243 for pH 7 and 10. Interestingly, pro-fragrance **3** did not show any measurable fragrance release at pH 7 and at pH 10 released the pro-fragrance only slowly (k = -0.051). In view of a previous report that showed faster release kinetics from hydrophilic as opposed to hydrophobic polymers for retro Michael addition,[13] this result is at first surprising. However, we assume that in the case of **3** the deprotonated transition state can be stabilized by intramolecular hydrogen bonding to the free hydroxyl groups of the α-D-galactopyranose moiety, which reduces the rate of the elimination step. The executed Nuclear Overhauser Effect (NOE) experiment did neither confirm nor deny our hypothesis of stabilization, which is not possible in the case of **2**, and which might explain the higher release rate of this motif.

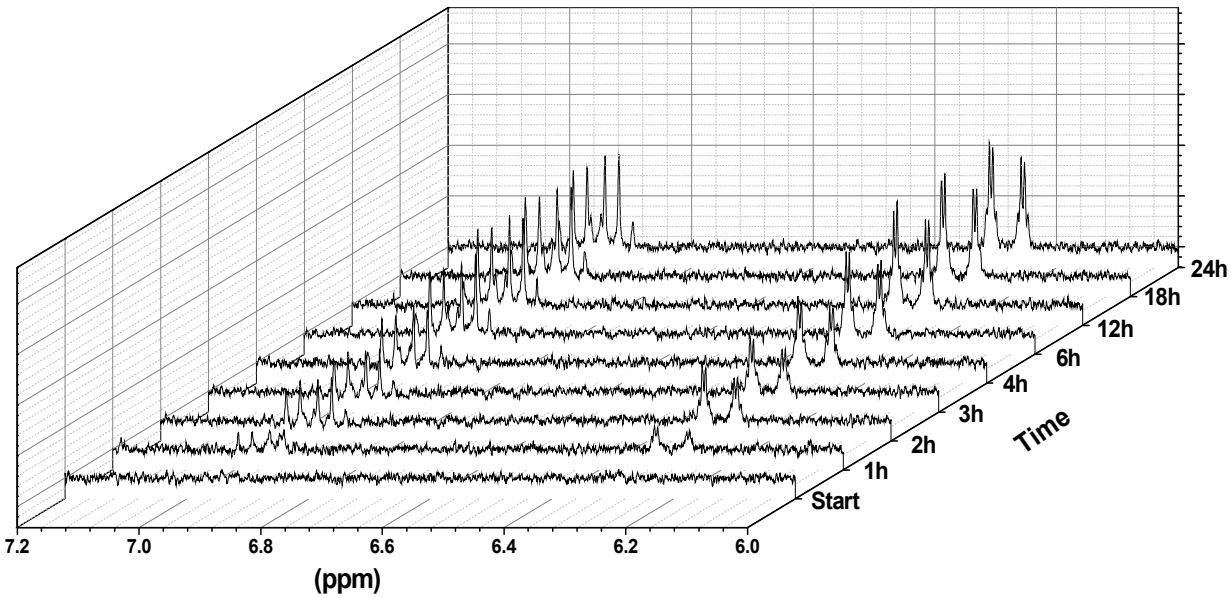


Figure 3. ^{1}H NMR spectra of pro-fragrance 2 in a mixture of deuterated methanol and a D_2O-based buffer at pH 10 as function of time. The emerging resonances at 6.96 and 6.28 ppm are diagnostic for the two enonic protons of released δ-damascone and were used to establish the release kinetics shown in Figure 4.

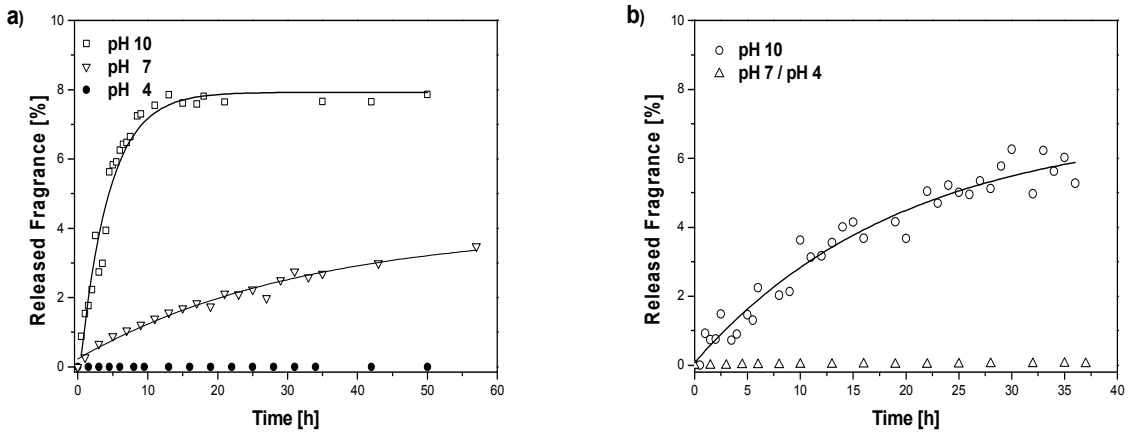
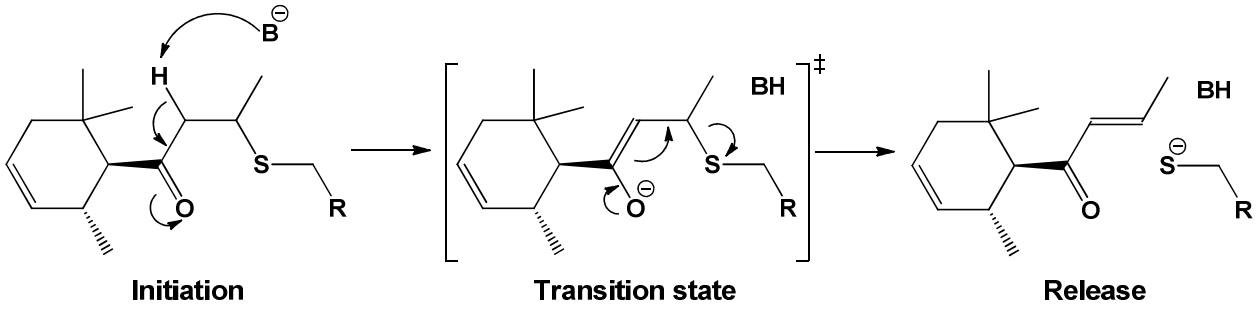


Figure 4. Amount of δ-damascone released from a) pro-fragrance **2** and b) pro-fragrance **3** in a mixture of deuterated methanol and a D_2O-based buffer at pH 4, 7, and 10 as function of time. The data were extracted from *in situ* ^{1}H NMR experiments (Figure 3). Solid lines represent best fits of Eq. 1 to the data.



Scheme 3. Release mechanism for retro Michael addition.

Dynamic headspace analysis for δ-damascone release from ceramic tiles

The δ-damascone release from the new pro-fragrances under ambient conditions was next studied using unpainted ceramic tiles (10 x 5 cm) as a substrate. This substrate permitted quantitative deposition of known amounts of the pro-fragrances, and is also relevant for the application in cleaning products. For this purpose, pro-fragrances **2** and **3** and as a reference also the neat δ-damascone were independently combined with a standardized surface cleaning detergent and placed directly onto the tiles. We note that the detergent was slightly basic (pH 9); since the ^{1}H NMR-studies (*vide supra*) showed an accelerated release for pro-fragrances **2** and **3** under basic aqueous conditions, some release of the payload must be expected under long-term storage. The tiles were dried for three days and dynamic headspace analysis was conducted according to the protocol reported previously.[13,35] In brief, the samples were placed into headspace chambers that had a controlled temperature of 25 °C, and air with a

constant humidity of 75% was passed over the sample (200 mL·min^{-1}). Volatiles were collected on specially designed absorbers, and subsequently thermally desorbed and analyzed by gas chromatography (GC) and mass spectroscopy (MS) (an example of the GC-MS spectra is shown in Supporting Figure S 10). The GC data were evaluated against calibration curves acquired with the neat δ-damascone, which permitted the construction of the release profiles shown in Figure 5.

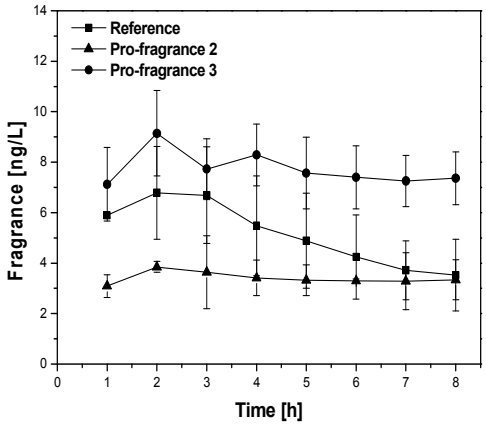


Figure 5. Concentration of δ-damascone released from pro-fragrances **2** and **3** and the neat δ-damascone in air passed over ceramic tiles onto which the respective pro-fragrances had been deposited. Samples were collected after drying the samples in the dark under ambient conditions for 3 days. Data points represent averages of two samples.

Figure 5 shows several interesting trends. First of all, the tiles treated with pro-fragrance **3** releases more fragrance than the one treated with the neat δ-damascone reference. In the case of **3** the release is stable for many hours (we re-iterate that at the beginning of the experiment the samples are already 3 days old), whereas a decrease is seen in the case of the reference treated with the neat δ-damascone. This effect is consistent with a rapidly decreasing fragrance concentration on/in the tile. The tiles used are porous; while the pores retain a significant amount of δ-damascone during drying at ambient, evaporation is greatly accelerated upon applying an airstream. Pro-fragrance **2** shows a low, but stable release. By and large the data seem to meet the expectations. A substantial fraction of the originally applied pro-fragrance **3**, which as the pH-dependent solution studies showed has the slower release kinetics, appears to be present in the original form and slowly releases a substantial amount of the fragrance over an extended period of time. In the case of the more labile pro-fragrance **2**, which has

probably has already partially decomposed during drying and the neat δ-damascone reference some of the fragrance has already evaporated and consequently smaller amounts are released.

Dynamic headspace analysis of δ-damascone release from washed cotton fabric

To test our hypothesis that the (protected) α-D-galactopyranose might, on account of similarity in chemical structure, be a good motif to physically anchor pro-fragrances onto cotton in under emulated application conditions, we added pro-fragrances **2** and **3** and as reference also the neat δ-damascone to a simplified fabric softener emulsion, which comprised a commercially available cationic surfactant (Stepantex® VK 90, a dialkylester ammonium methosulfate) and which had a pH of about 3.1. The acidic nature is favorable for storing pro-fragrances which uses the retro 1,4-addition for the release mechanism as it has already been shown in the past.[16,32] Another well-known effect of the surfactant, besides acting as a softener which smoothens the fabric, is its ability to facilitate the deposition of nonpolar molecules onto the cotton surface.[24] Thus, cotton sheets were washed with aqueous mixtures comprising the surfactant and either of the pro-fragrances, wringing the wet tissue to a pre-defined weight, and drying the samples for three days in a dark cupboard (Figure 1). Overall, this process simulates the washing, drying, and storage of clothes. After drying was complete, the release of δ-damascone was probed by dynamic headspace analysis as discussed before; the results are summarized in Figure 6. The data reveal that cotton sheets washed with the pro-fragrances have a much higher release rate than the sample treated with the unmodified δ-damascone. Pro-fragrance **2** releases up to 55 $ng \cdot L^{-1}$, which is five times more than pro-fragrance **3** and 130 times more than the neat δ-damascone reference. Both pro-fragrances release the fragrance, which has an olfactory threshold of 0.021 $ng \cdot L^{-1}$ air, in concentrations well above the human detection limit.[36] The release profiles of the two pro-fragrances have similar shapes, and are characterized by an initial increase, which is a typical feature that has been related to equilibration,[37] before the release rate appears to level off at value that is at least constant for several hours. As will be demonstrated by the experiment that follows, the much higher absolute release rate of **2** is due to better adsorption of this hydrophobic pro-fragrance on cotton than the more polar pro-fragrance **3** (at least under the deposition conditions chosen here), which translates into a larger amount of the pro-fragrance that is deposited on the substrate during the washing process.

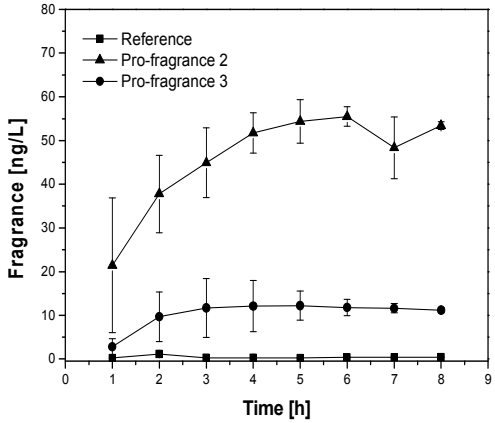


Figure 6. Concentration of δ-damascone released from pro-fragrances **2** and **3** and the neat δ-damascone in air passed over cotton tissues that had been washed with aqueous mixtures comprising the surfactant and either of the pro-fragrances and drying the samples for three days in a dark cupboard. Data points represent averages of two samples.

Dynamic headspace analysis of δ-damascone released after direct deposition onto cotton tissue

To separate the effect of deposition from the effect of release seen in the softener washing release tests (SW), the pro-fragrances were also directly deposited (DD) onto cotton fabric surface and analyzed via headspace analysis. Comparing the softener washing method with the direct deposition, a deconvolution of the effect of deposition and fragrance release was possible. The comparison between direct and indirect deposition is also very important to determine the cost to performance ratio.

For this, a cotton tissue was washed in the softener emulsion without any added fragrance, and the pro-fragrance was directly applied onto the material. For a control sample, an equimolar amount of δ-damascone was added to a separate square of fabric prepared in the same manner. For example, 24 mg of pro-fragrance **2,** 20 mg of pro-fragrance **3** and 8.7 mg of neat δ-damascone were dissolved in separate aliquots of 200 µL of ethanol and put onto the freshly washed and wrung cotton tissues. These weights correspond to equal amounts of potentially releasable fragrance. These tissues were than line dried in a dark cupboard for three days. After three days the above described headspace analysis was performed and the data is shown compared to the aqueous softener simulation in Figure 7.

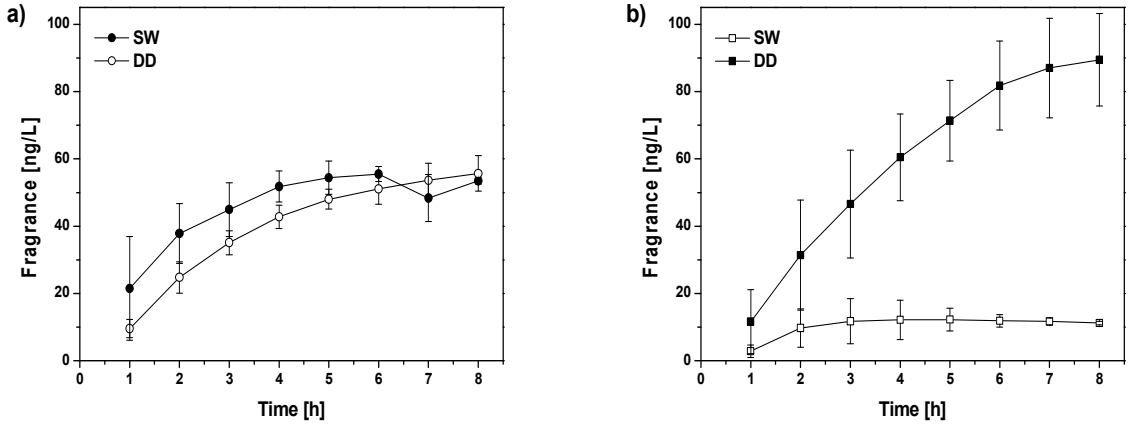


Figure 7. Comparison of released δ-damascone (in ng L^{-1} of air) from cotton tissue after three days of drying for softener wash (SW) and direct deposition (DD) a) pro-fragrance **2** and b) pro-fragrance **3**. Data points represent averages of two samples.

The apolar pro-fragrance **2** shows nearly an identical amount of released fragrance in both the softener wash and in the direct deposit method. This suggests that the deposition rate of apolar compounds is very efficient, which could be attributed to the polar environment of the softener washing step. Presumably, the apolar pro-fragrance **2** is preferentially deposited onto the cotton tissue, as it has been described for polymeric pro-fragrances.[13] In contrast, pro-fragrance **3** shows much better release from direct deposition as compared to the softener washed samples. This can be attributed to the higher hydrophilicity of pro-fragrance **3**, thus a higher solubility in the washing solution, and subsequently less is deposited on the sample.

Gratifyingly, a higher release rate after 3 days of pro-fragrance **3** suggests slower release kinetics for the more polar compounds, confirming previously hypothesis of the authors.[13] This is at first glance, at odds with NMR-studies, which show the opposite result. However, this presumably can be attributed to the fact that the NMR-studies are done in a liquid environment while the deposition is monitored from a dried surface. This higher release could be due to the hygroscopic behavior of pro-fragrance **3**, which is easily observable by keeping it non closed vial on the bench overnight. As the release mechanism needs a proton source, the humidity of the air combined with the hygroscopic nature of pro-fragrance **3** could be the reason for the better release instead of the less hygroscopic and apolar pro-fragrance **2**. In creating this polar environment the release from dry cotton is accelerated.

CONCLUSIONS

Carbohydrate molecules are interesting materials when it comes to enhance the evaporation time of fragrances and to introduce adhesion to substrates. Our pro-fragrances were effective in two realistic application test environments, the softener and the detergent experiment. The tile release test showed that the deprotected pro-fragrance releases twice as much fragrance after 3 days than the corresponding protected pro-fragrance and the reference scent. The protected analog did not show a significant difference compared to the reference. NMR studies of the retro 1,4-addition, releasing the fragrance, showed an accelerated release in a basic environment mimicking the cleaning conditions. The performance of our pro-fragrances in the softener test showed a much higher release of fragrance compared to the reference, most likely due to the acidic environment of the softener composition. The pro-fragrances stay stable under acidic conditions and prolong the release period of δ-damascone up to 130 times for pro-fragrance **2** compared to the reference molecule. It was shown that not only the release period was extended, but also the deposition rate of the pro-fragrances onto cotton tissue could be improved. The protected and therefore more hydrophobic pro-fragrance **2** gave the best results in the softener release test. This observation is also supported by earlier studies for apolar molecules[24] and polymers[13] where the deposition of hydrophobic compounds is more efficient than the one for hydrophilic ones. Taking in account the release data from the direct deposition, pro-fragrance **3** shows the highest release after three days, due to the more hydrophilic nature of the backbone molecule.

By slightly modifying the polarity of the sugar moiety we are able to target two different applications for these pro-fragrances. Pro-fragrance **2** could be used to extend the smell of washed clothes. In contrast pro-fragrance **3** is much more effective when directly deposited onto the target area, which should be an opportunity for extending the fragrance smell in body care, cleaning and many other consumer products.

Supporting Information Available:

NMR, IR, Buffer preparation as described in the text. This material is available free of charge via the Internet at http://pubs.rsc.org.

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Notes :

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Alain Trachsel (Firmenich SA) for the help with the headspace analysis and GC-MS measurements. The authors gratefully acknowledge Firmenich SA for financial support.

REFERENCES

1. H. Surburg and J. Panten, *Common Fragrance and Flavor Materials*, Wiley-VCH, Weinheim, 2006.
2. E. Breitmaier, *Terpenes - Flavors, Fragrances, Pharmaca, Pheromones*, Wiley-VCH, Weinheim, 2006.
3. G. Ohloff, W. Pickenhagen and P. Kraft, *Scent and Chemistry*, Wiley-VCH, Weinheim, 2011.
4. E. Pichersky, J. P. Noel and N. Dudareva, *Science*, 2006, **311**, 808–811.
5. J.-E. Sarry and Z. Günata, *Food. Chem.*, 2004, **87**, 509–521.
6. R. Ciriminna and M. Pagliaro, *Chem. Soc. Rev.*, 2013, **42**, 9243–9250.
7. E. A. Stefanescu and C. Stefanescu, *Mater. Sci. Res. J.*, 2012, **6**, 43–102.
8. G. Y. Zhu, Z. B. Xiao, R. J. Zhou and F. P. Yi, *Adv. Mater. Res.,* 2012, **535-537**, 440–445.
9. K. Landfester and C. Weiss, in *Modern Techniques for Nano- and Microreactors/-reactions*, ed. F. Caruso, Springer Berlin Heidelberg, 2010, vol. 229, pp. 1–49.
10. A. Herrmann, *Angew. Chem. Int. Ed.*, 2007, **46**, 5836–5863.
11. A. Herrmann, *Photochem. Photobiol. Sci.*, 2012, **11**, 446–459.
12. V. Rataj, F. Ruyffelaere and J. M. Aubry, *Cah. Formulation*, 2005, **12**, 82–96.
13. D. L. Berthier, N. Paret, A. Trachsel and A. Herrmann, *Bioconjugate Chem.*, 2010, **21**, 2000–2012.
14. U. Maddalena, A. Trachsel, P. Frankhauser, D. Berthier, D. Benczédi, W. Wang, X. Xi, Y. Shen and A. Herrmann, *Chem. Biodivers.*, 2014, **11**, in press (DOI: 10.1002/cbdv.201400023).
15. H. Kamogawa, H. Mukai, Y. Nakajima and M. Nanasawa, *J. Polym. Sci., Polym. Chem. Ed.*, 1982, **20**, 3121–3129.
16. D. Berthier, A. Trachsel, C. Fehr, L. Ouali and A. Herrmann, *Helv Chim Acta*, 2005, **88**, 3089–3108.
17. H. Morinaga, H. Morikawa, Y. M. Wang, A. Sudo and T. Endo, *Macromolecules*, 2009, **42**, 2229–2235.
18. A. Trachsel, J. Y. de Saint Laumer, O. P. Haefliger and A. Herrmann, *Chem. Eur. J.*, 2009, **15**, 2846–2860.
19. Y. Wang, H. Morinaga, A. Sudo and T. Endo, *J. Polym. Sci., Part A: Polym. Chem.*, 2010, **48**, 3816–3822.
20. H. Morinaga, H. Morikawa, A. Sudo and T. Endo, *J. Polym. Sci., Part A: Polym. Chem.*, 2010, **48**, 4529–4536.
21. D. Berthier, N. Paret, A. Trachsel, W. Fieber and A. Herrmann, *Polymers*, 2013, **5**, 234–253.
22. G. J. Mahler, M. B. Esch, E. Tako, T. L. Southard, S. D. Archer, R. P. Glahn and M. L. Shuler, *Nat. Nanotechnol.*, 2012, **7**, 264–271.
23. W. C. E. Schofield and J. P. S. Badyal, *ACS Appl. Mater. Interfaces*, 2011, **3**, 2051–2056.
24. S. K. Obendorf, H. Liu, K. Tan, M. J. Leonard, T. J. Young and M. J. Incorvia, *J Surfactants Deterg.*, 2009, **12**, 43–58.
25. A. Leo, C. Hansch, D. Elkins, *Chem. Rev.* 1971, **71**, 525–616.
26. A. J. Leo, *Chem. Rev.* 1993, **93**, 1281–1306.

27. T. Ikemoto, K. Mimura and T. Kitahara, *Flavour Fragrance J.*, 2003, **18**, 45–47.
28. T. Ikemoto, B. Okabe, K. Mimura and T. Kitahara, *Flavour Fragrance J.*, 2002, **17**, 452–455.
29. O. R. Jensen and F. Okkels Thyge (to Poalis A/C), PCT Int. Appl. WO 2006/087370, 2006.
30. D. Milotic, *J. Consum. Behav.*, 2003, **3**, 179–191.
31. S. Zeng, S. Sun, S. Liu, J. Hu and B. He, *J. Therm. Anal. Calorim.*, 2013, **115**, 1049–1056.
32. C. Fehr and J. Galindo, *Helv. Chim. Acta*, 2005, **88**, 3128–3136.
33. A. Gille and M. Hiersemann, *Org. Lett.*, 2010, **12**, 5258–5261.
34. W. R. Carmody, *J. Chem. Educ.*, 1961, **38**, 559–560.
35. A. Trachsel, C. Chapuis and A. Herrmann, *Flavour Fragrance J.*, 2013, **28**, 280–293.
36. D. Kastner, *Parfuem. Kosmet.*, 1994, **75**, 170–181.
37. S. R. Steinfeld, J.S. Francisco; W. L. Hase, *Chemical kinetics and dynamics*, Prentice Hall, Upper Saddle River, 1998.

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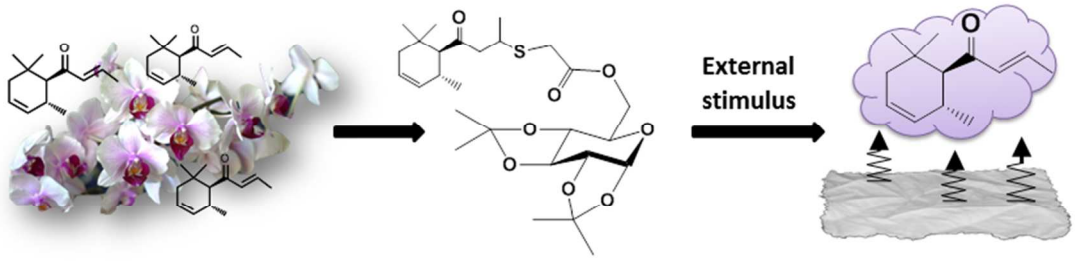

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