Enzymatic Modification of Palmarosa Essential Oil: Chemical Analysis and Olfactory Evaluation of Acylated Products

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We have developed an enzymatic protocol to modify the composition of palmarosa essential oil by acylation of its alcohol components by three different acyl donors at various rates. The resulting modified products were characterized by qualitative and quantitative analyses by gas chromatography, and their olfactory properties were evaluated by professional perfumers. We showed that our protocol resulted in two types of modifications of the olfactory properties. The first and most obvious effect observed was the decrease of the alcohol content, with the concomitant increase of the corresponding esters, along with their fruity notes (pear, most notably). The second and less obvious effect was the expression of notes from minor components ((*E*)-β-ocimene, linalool, β-caryophyllene, and farnesene), originally masked by the sweet-floral-rose odor of geraniol, present in 70% in the palmarosa essential oil used, and emergence of citrus, green, spicy and clove characters in the modified products. This methodology might be considered in the future as a sustainable route to new natural ingredients for the perfumer.

Introduction. – The use of enzymes in the synthesis of fine chemicals, from laboratory to industrial scale, is now in many instances a competitive alternative to conventional synthesis [1–5], with advantages such as the control of the chirality of the products [6][7], the mild operating conditions, and the overall sustainable aspects of the processes [8][9]. Such trends are also observed in the field of flavors and fragrances, with the additional advantage of providing transformations eligible to the valuable 'natural' label [10–13]. Existing enzyme-based methodologies typically deal with single-molecule manipulation [14–16]. On the other hand, essential oils of various origins, and more generally, odoriferous natural extracts have been used in fragrances since perfumery was born. As a consequence of their complex composition, their olfactory impact on perfuming compositions is also complex [17]. Today, modern perfumery uses both essential oils and synthetic materials, and innovation in fragrance chemistry is focused on the use of new sources of natural extracts [18], new techniques of extraction [19–21], and the design of novel synthetic odorants [22]. However, the status of essential oil alone does not guarantee the access to the perfumer's shelf, and superior olfactory properties are necessary. As a result, the price range for essential oils can vary significantly from a few Euros to more than 10,000 per kg, depending on supply and demand.

In this context, we have been interested in providing new ingredients for the perfumer by modifying essential oils with enzymes. In particular, we became interested

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in using simple enzymatic transformations (*e.g.*, lipase-based acylation of alcohols) to modify inexpensive and available essential oils such as palmarosa essential oil, and to evaluate the subsequent changes of olfactory properties. Two questions, however, had to be addressed: *1*) what will be the global effect of these multiple changes on the odor of the mixture, which results in complex relationships between odorants in the matrix, and *2*) how interesting will be these modified extracts as new materials for the perfumers.

Results and Discussion. – To our knowledge, the use of enzymes in essential-oil research has been limited so far to the pectinase/cellulase-assisted extraction from bergamot (*Citrus bergamia* Risso) peel [23], congolese mansa (*Solanum americanum* Miller) [24], rose (*Rosa* sp.) [25–27], olive [28–30], lemon peel [31], lemongrass (*Cymbopogon citratus* Stapf.), and lemon eucalyptus (*Eucalyptus citriodora* Hook) [32]. We recently reported the use of an enzymatic strategy based on the use of horseradish peroxidase (HRP) for the removal of eugenol from rose essential oil without loss of the organoleptic quality and with a good conservation of the chemical composition [33]. For the first time, an enzyme-based strategy was proposed for essential-oil treatment in a model study of the detoxification of natural extracts used in perfumery and cosmetics.

In the present work, we used the enzymatic modification strategy to chemically modify an essential oil from palmarosa, rich in geraniol and exhibiting, therefore, strong sweet-floral-rose notes with lemongrass and straw undertones, and improve its olfactory quality with the aim of providing new materials for the perfumers. Previously, the lipase-catalyzed acetylation of palmarosa by AcOH to obtain natural geranyl acetate was reported, but the nature of the enzyme was not disclosed, and neither the chemical modifications nor the olfactory impact of the modification were investigated [34].

Lipase-Catalyzed Acylation of Palmarosa Essential Oil. The lipase-catalyzed acylation of palmarosa essential oil was performed using *Candida rugosa* lipase and following a procedure developed in toluene on similar compounds (*Scheme 1*) [35].

Using vinyl (ethenyl) esters as acyl donors towards palmarosa essential oil, several products featuring various yields of esterification could be obtained by varying the amount of acyl donor used combined with the application of different reaction times (*Table 1*).

In general, various yields of acylation could be obtained ranging from 20 to 100% by using 0.25–3 equiv. of acyl donors involved and in 27–168 h reaction time. The amounts of the isolated modified essential oils were usually good (33–50 mg),

Scheme 1. *Example of Acylation of Geraniol by* n *Equivalent of Acyl Donor* (*n* 0.25–3) *catalyzed by* Candida rugosa *lipase* (*CrL*)

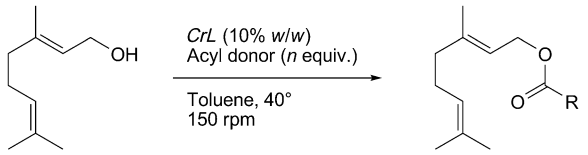
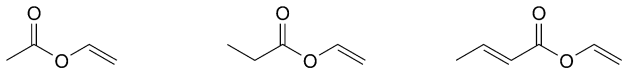


Table 1. *Acylation of Palmarosa Essential Oil by Vinyl Esters Catalyzed by* Candida rugosa *Lipase*



Vinyl acetate (V.A.) Vinyl propionate (V.P.) Vinyl crotonate (V.C.)

Entry[a])	Acyl donor	Equiv.	Reaction time [h]	Amount of modified oil obtained [mg]	Acylation yield [%][b])
1	V.A.	0.25	27	33	24
2	V.A.	0.5	27	35	38
3	V.A.	1.5	121	37	80
4	V.A.	3	168	33	100
5[c])	V.P.	0.75	88	40	77
6	V.P.	2	88	50	100
7	V.C.	0.2	73	49	20
8	V.C.	0.45	73	36	40
9	V.C.	1.5	73	48	84
10	V.C.	3	168	37	100

[a]) Conditions: palmarosa essential oil (50 mg) in toluene (5 ml); temp., 40°, rotation in an incubator, 150 rpm. [b]) Based on the acylation of citronellol performed in parallel and determined by ^{1}H-NMR of the crude mixture. [c]) Performed in duplicates (1.3% relative error).

relatively to the amount of starting material (50 mg) with a little loss during workup involving evaporation steps.

Gram-Scale Experiment. Selected reactions could be scaled-up by a factor of 10 starting with 0.5 g of palmarosa essential oil without noticeable loss of efficiency. The results are compiled in *Table 2*.

Table 2. *Examples of Acylation of Palmarosa Essential Oil by Vinyl Esters Catalyzed by* Candida rugosa *Lipase at the Gram Scale*

Entry[a])	Acyl donor[b])	Equiv.	Reaction time [h]	Amount of modified oil obtained [mg]	Acylation yield[c]) [%][c])
1	V.A.	1	24	410	>46
2	V.A.	1	96	422	100
3	V.C.	1	72	393	58

[a]) Conditions: Palmarosa essential oil (500 mg) in toluene (5 ml); temp., 40°; rotation in an incubator, 150 rpm. [b]) See *Table 1*. [c]) Based on the acylation of citronellol performed in parallel and determined by ^{1}H-NMR of the crude mixture.

Modified essential oils could thus be obtained on this scale according to a similar procedure to obtain partially acylated products, at a yield of 46 and 58% with vinyl acetate and vinyl crotonate (but-2-enoate), respectively (*Entries 1* and *3*), and a 100% acetylated product with a longer reaction time (96 h; *Entry 2*).

Vinyl esters as acyl donors are very efficient partners in this type of reactions. Indeed, the enol eliminated being equilibrated to the non-nucleophile acetaldehyde,

total acylation can be achieved. However, in terms of sustainability of the process and naturality of the products, other acyl donors should be preferred. We have thus investigated the use of natural AcOH and AcOEt as acyl donors in a model reaction on citronellol. Following results recently described in the literature by others, we first focused on the direct use of AcOH as donor [34]. In spite of a reported esterification yield of 52.5%, obtained after heating an equimolar mixture of geraniol and AcOH during 34 h at 60°, no conversion of citronellol was observed under the same conditions with *Candida rugosa* lipase or *Candida antarctica* lipase B, and the starting material was recovered unchanged in 90–96% yield. We next turned our attention to the possible use of AcOEt, satisfactorily involved as acyl donor in the acylation of tyrosol by *Staphylococcus xylosus* lipase [36]. A screening of the conditions was performed, and the results are collected in *Table 3*.

Table 3. *Screening of the Reaction Conditions for the Acylation of Citronellol by AcOEt in the Presence of* Candida rugosa *Lipase*

Entry[a])	AcOEt [equiv.]	Conversion [%]	Enzyme/substrate (*w*/*w* [%])	Acylation yield[b]) [%]
1	3	33	10	5
2	5	37	10	5
3	3	42	20	10
4	160[c])	60	10	10
5	160[c])	37	20	15
6	160[c])	59	50	32
7	160[c])	62	75	50
8	160[c])	70	100	66
9	160[c])	100[d])	60	100

[a]) Conditions: citronellol (50 mg) was dissolved in 10 ml of anh. toluene and incubated 24 h at 150 rpm at 40° in the presence of *n* equivalent(s) of AcOEt and 5 mg of lipase. [b]) Determined by ^{1}H-NMR. [c]) AcOEt was used as the solvent. [d]) Reaction time of 96 h was required.

Under the conditions previously determined with vinyl esters, the use of AcOEt as acyl donor did not lead to satisfactory acylation yields (*Entries 1–3*). Even using AcOEt as solvent, only 10% acylation was observed in 24 h in the presence of 10% (*w*/*w*) lipase (*Entry 4*). The acylation yield increased linearly as a function of the amount of enzyme used, as a result of the saturation of the enzyme by the substrate (*Entries 4–8*). Optimized conditions were found with 60% lipase and a reaction time of 96 h to obtain 100% acylation yield (*Entry 9*). This last result indicated that a biocatalytic route to 'natural modified' palmarosa essential oil could be envisaged.

Olfactory Evaluation. The olfactory evaluation of the modified palmarosa essential-oil samples was performed by professional perfumers. In addition to the samples obtained from acylation reactions, untreated essential oil was evaluated, as well as a negative control sample consisting in the essential oil submitted to the entire procedure without enzyme. The results are compiled in *Table 4* with details for each sample.

Qualitative and Quantitative GC Analyses. The qualitative and quantitative chemical compositions of the untreated palmarosa essential oil, the negative control

Table 4. *Olfactory Evaluation of the Modified and Unmodified Palmarosa Essential Oils*

Entry	Sample	Acylation rate [%]	Notes	Interest, originality[a])
1	Untreated	–	Rose-like, lemongrass, straw	N.A.
2	Neg. control[b])	–	Rose-like, citrus, metal	1.0
3	Acetylated	24	Citrus, metal, straw, lemon	3.3
4	Acetylated	38	Rose-like, geranium, floral, citrus	5
5	Acetylated	80	Lemon, spicy, eugenol, frutty, pear, fresh	3
6	Acetylated	100	Pear, green, spicy	3
7	Propanoylated	76	Pear, fruity, plastic, rose-like	3
8	Propanoylated	100	Fruity, marine, pear, floral	3.3
9	Crotonylated	20	Fruity, plastic, burnt	2.5
10	Crotonylated	40	Apple, rose-like, weak	1.5
11	Crotonylated	84	Plastic, nutty, straw, floral, fruity	1
12	Crotonylated	100	Burnt, plastic, animalic	1

[a]) From 1 (no interest) to 5 (very interesting). N.A.: not applicable. [b]) Negative control consisting in palmarosa essential oil submitted to the entire procedure without enzyme.

without enzyme, and each modified sample was analyzed by combined GC/MS and GC-FID analyses (*Table 5*).

In *Table 5*, we have collected the most representative samples with modified composition. We could identify 50 compounds accounting for 95.8–100% of the total chromatogram area. The other results are available as *Supplementary Information*. The comparison between untreated essential oil and the negative control, consisting in submitting the essential oil to the whole reaction process in the absence of enzyme, showed no significant difference. This result was very important to show that the differences observed in the modified samples are of enzymatic origin, and that the mass loss by evaporation does not affect the quantitative composition. Besides the expected ester (acetate, propanoate, and crotonate), longer chain esters were also identified in the modified essential oils. The formation of these compounds could be explained by the presence of their vinyl analogs as impurities in the starting vinyl esters (purity 99, 98, and 98% for V.A., V.P., and V.C., resp.).

As a result of the action of *Candida rugosa* lipase in the presence of acyl donors, the geraniol contents, as well as other similar alcohols, decreased, and the corresponding esters were formed, with increased fruity notes. Interestingly, the decrease in geraniol in the modified samples allowed other compounds, present at low percentages, to express their own odor, as identified in the sensory analysis (*Table 4*). It is the case, for example for (*E*)-β-ocimene, farnesene, and linalool, with citrus and green notes (appearing in the acetylated products at 24 and 38%, resp.), β-caryophyllene with spicy-woody-clove character (see acetylated sample at 80% with eugenol undertone), and obviously geranyl acetate with the rose-pear-floral notes and farnesyl acetate with green-floral-rose notes, both heavily felt in the sensory analysis of the acetylated sample at 100%. Transformations of these impact odorants into each other could not be neglected, since C_{10}- and C_{15}-derived compounds are related by simple organic transformations that could occur in the reaction medium (*Scheme 2*).

Table 5. *Chemical Composition of Untreated and Selected Acylated Palmarosa Oils*

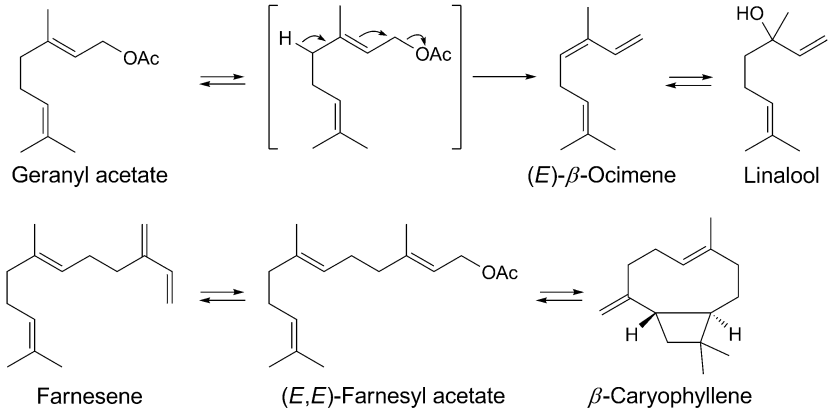
Constituents	*RI*		Samples[a])							
	Apolar	Polar	Untreated	Neg. ctrl.	V.A. (24%)	V.A. (38%)	V.A. (80%)	V.A. (100%)	V.P. (100%)	V.C. (100%)
6-Methylhept-5-en-2-one	966	1342	0.1	–	–	–	–	–	–	–
Myrcene	984	1162	0.3	0.3	0.1	0.1	0.1	0.1	0.2	0.1
Limonene	1024	1193	0.2	0.2	–	–	–	t	–	–
(*Z*)-*β*-Ocimene	1030	1239	0.5	0.5	t	0.1	0.1	t	t	0.1
(*E*)-*β*-Ocimene	1042	1253	1.7	1.5	0.1	0.3	0.1	t	0.1	0.1
(*Z*)-Linalol oxid (furanoid)	1063	1425	–	0.1	t	–	–	–	–	–
(*E*)-Linalol oxid (furanoid)	1075	1451	–	–	t	–	–	–	–	–
Linalool	1088	1562	4.6	4.2	1.2	1.2	0.7	0.8	0.7	0.5
Allocimene	1117	1387	0.1	–	–	–	–	–	–	–
Citronellal	1134	1479	t	–	–	–	–	–	–	–
Nerol oxide	1136	–	t	–	–	–	–	–	–	–
α-Terpineol	1175	1708	–	–	–	–	–	t	–	–
Nerol	1210	1788	–	t	0.1	–	–	–	–	0.1
Citronellol	1217	1788	–	–	–	–	t	–	t	–
Neral	1217	1676	0.6	0.6	0.3	0.1	0.2	t	0.2	0.2
Geraniol	1245	1879	69.1	68.7	57	37.5	0.2	0.2	0.1	0.1
Geranial	1248	1743	–	–	–	–	1.3	2.3	0.4	0.6
Neryl formate	1271	1708	0.1	0.1	–	–	–	–	–	–
Geranyl formate	1284	1688	0.2	0.2	0.9	0.1	0.4	0.1	0.1	0.1
Unknown compound[c])	1310	–	0.3	0.3	0.1	0.1	–	–	–	–
Citronellyl acetate	1334	1655	–	–	–	–	0.1	t	–	–
Neryl acetate	1342	1743	0.1	–	0.1	0.1	0.8	0.2	–	–
Geranyl acetate	1362	1763	12.9	12.7	29.7	54.3	90	89	9.4	10.6
β-Elemene	1389	1602	–	–	0.1	0.1	–	0.1	0.1	0.1
β-Caryophyllene	1422	1584	3.3	3.1	1.2	1.4	1.4	2.3	1.9	2.1
Citronellyl propionate	1429	1705	–	–	–	–	–	–	0.8	–
Neryl propionate	1430	–	–	–	–	–	–	–	0.3	–
2,3-Epoxygeranyl acetate	1435	–	–	–	t	0.1	t	–	–	–
Geranyl propionate	1446	1823	–	–	–	–	–	–	80.1	–
α-Humulene	1460	1688	0.2	0.2	0.1	0.1	t	0.2	–	0.2
(*E*)-*β*-Farnesene	1460	1688	0.1	0.1	0.2	–	0.2	0.3	–	–

Table 5 (cont.)

Constituents	*RI*		Samples[a])							
	Apolar	Polar	Untreated	Neg. ctrl.	V.A. (24%)	V.A. (38%)	V.A. (80%)	V.A. (100%)	V.P. (100%)	V.C. (100%)
Guaiarome	1476	–	–	–	0.1	0.1	–	–	–	0.1
β-Selinene	1480	1743	0.1	–	–	–	–	0.1	–	–
α-Selinene	1484	1788	t	–	–	–	0.2	0.1	0.1	–
Valencene	1485	–	0.1	–	0.1	0.1	t	0.1	–	t
7-Epi-α-santalene	1519	–	–	–	–	–	–	–	0.1	–
Geranyl butyrate	1532	1923	0.4	0.5	0.5	0.3	0.5	0.1	0.3	0.3
Nerolidol	1540	2030	0.3	0.3	0.3	0.3	0.3	0.3	0.4	–
Caryophyllene oxide	1575	1994	0.6	0.5	0.3	0.3	0.2	0.4	0.6	0.6
Geranyl isovalerate	1580	–	0.1	–	–	–	0.2	0.1	–	–
Geranyl crotonate	1593	2020	–	–	–	–	–	–	–	80.1
Humulene oxide	1594	–	–	–	0.3	–	–	–	–	–
Farnesol	1656	2275	1.6	1.2	1	0.9	0.1	0.1	–	0.5
Geranyl caproate	1722	–	–	–	–	–	–	–	1.2	–
Geranyl hexanoate	1723	2071	–	1.2	0.6	0.9	0.2	0.6	-	1.1
(*Z*,*E*)-Farnesyl acetate	1820	2202	–	0.1	0.5	0.9	1.5	0.9	1.9	0.1
Geranyl octaonate	1927	–	–	–	0.6	–	–	1.4	–	–
Geranyl valerate	1922	–	t	0.7	–	–	0.2	–	–	–
Farnesal	1711	2287	t	–	0.1	–	–	–	–	–
Geranyllinalool	2321	2321	0.1	t	0.1	0.1	0.1	0.1	–	–
Unidentified			2.3	2.5	4.2	0	0.8	0.1	0.7	2

[a]) The modified samples are labelled with the acyl donor (V.A., V.P., and V.C.) and the yield of acylation in parenthesis; tr., trace. [b]) EI-MS (70 eV): 155 (1); 152 (1); 136 (4); 121 (8); 109 (100); 95 (33); 82 (64); 69 (98); 61 (31); 55 (40); 41 (96).

Scheme 2. *Possible Relationships between Key Compounds Varying in the Series of Modified Essential Oils Considered*



Vinylogous elimination of the AcO of geranyl acetate could indeed form (*E*)-*β*-ocimene, which leads to linalool after *Markovnikov*-selective hydration. Similarly, farnesyl acetate could lead to farnesene, and further to *β*-caryophyllene by cyclization. It is worth noting that (*E*,*E*)-farnesyl pyrophosphate is the biosynthetic precursor of *β*-caryophyllene [37].

Conclusions. – In summary, we have developed a lipase-based strategy to gently modify palmarosa essential oil and allow the expression of different olfactory notes from the esters formed, but also from odorants originally present but inhibited by the large quantity and impact of geraniol. Detailed qualitative and quantitative chemical analyses have been performed and allowed characterization of the slight differences between all the modified samples, besides the formation of geranyl esters. This type of modification might be considered in the future to develop new ingredients for fine perfumery.

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Experimental Part

General. Chemicals such as citronellol and vinyl esters, and the org. solvents were purchased from *Sigma–Aldrich*, and used as received, except toluene, which was dried over $CaCl_2$ and distilled at atmospheric pressure. Enzymes were purchased from *Sigma–Aldrich* (*Fluka*) and stored at −18°. Palmarosa essential oil from India was a kind gift of *Payan Bertrand S.A.* (F-Grasse). Anal. TLC: 0.2-mm precoated plate silica gel *60 F254* (*Merck*). ^{1}H- and ^{13}C-NMR spectra: *BRUKER AC 200*, chemical shifts (δ) in ppm relative to TMS at 0 ppm (1 H) or $CDCl_3$ (77.16 ppm; ^{13}C); coupling constants, *J*, in Hz. GC/MS: *Shimadzu QP2010S-MS* chromatograph (EI; 70 eV) equipped with a *SLB-5ms* cap. column (thickness, 0.10 μm; length, 15 m; inside diameter, 0.10 mm), temp. program: 80°, then 18°/min to 200°, and maintained at this temp. for 30 min.

Acylation of Citronellol. In a 20-ml vial, citronellol (0.32 mmol, 50 mg) and the desired acyl donor (0.96 mmol) were dissolved in toluene (5 ml), and the lyophilized enzyme (*Candida rugosa* lipase; 5 mg) was then added. The vial was capped and the mixture was incubated at 40° and shaked at 150 rpm until completion, (monitoring by TLC). Workup consisted in decantation, withdrawal of the supernatant, removal of toluene *in vacuo*, liquid/liquid extraction with Et_2O/aq. 5% Na_2CO_3, collection of the org. phase, drying ($MgSO_4$), filtration, and solvent removal. Citronellyl acetate, propanoate, and crotonate were compared with original standards or with literature data [35].

Citronellyl Propionate. ^{1}H-NMR (200 MHz, $CDCl_3$, 20°): 5.10–4.90 (*m*, 1 H); 3.99 (*dt*, *J* = 1.8, 6.3, 2 H); 2.14 (*q*, *J* = 7.5, 2 H); 2.00–1.70 (*m*, 2 H); 1.70–1.10 (*m*, 11 H); 1.01 (*t*, *J* = 7.5, 3 H); 0.79 (*d*, *J* = 6.3, 3 H). ^{13}C-NMR (75 MHz, $CDCl_3$, 20°): 174.9; 131.7; 125.0; 63.2; 37.4; 35.9; 29.9; 28.0; 26.1; 25.8; 19.8; 18.0; 9.5. EI-MS (70 eV; selected ions): 212 (0, M^+), 138 (45), 123 (81), 109 (39), 95 (95), 81 (100), 69 (85), 57 (81), 41 (66).

Geranyl Propionate. ^{1}H-NMR (200 MHz, $CDCl_3$, 20°): 5.50–5.30 (*tq*, ^3J = 7.2, ^4J = 1.2, 1 H); 5.20–5.00 (*m*, 1 H); 4.66 (*d*, *J* = 7.2, 2 H); 2.39 (*q*, *J* = 7.6); 2.20–2.00 (*m*, 4 H); 1.76 (*m*, 6 H); 1.67 (*s*, 3 H); 1.20 (*t*, *J* = 7.6, 3 H). ^{13}C-NMR (75 MHz, $CDCl_3$, 20°): 174.9; 142.5; 132.2; 124.2; 118.8; 61.7; 39.9; 28.0; 26.7; 26.1; 18.1; 16.8; 9.6. EI-MS (70 eV; selected ions): 210 (1, M^+), 136 (18), 121 (27), 93 (36), 80 (16), 69 (100), 57 (57).

Quantitative GC Analysis. GC Analyses were carried out with an *Agilent 6890N* gas chromatograph, under the following operation conditions: vector gas, He; injector and detector temps., 250°; injected volume, 0.2 µl; split ratio 1/100; *HP1* column (*J&W Scientific*), polydimethylsiloxane (50 m × 0.20 mm i.d., film thickness, 0.33 µm; constant flow, 1 ml/min.) and *INNOWAX* (polyethyleneglycol, 50 m × 0.20 mm i.d. × film thickness 0.4 µm; *Interchim*, F-Montluçon). Temp. program: 45–250° at 2°/min and 250° for 60 min. Retention indices (*RI*s) were determined with C_5 to C_{24} alkane standards as reference. Relative amounts of individual components are based on peak areas obtained without FID response factor correction. Three replicates were performed for each sample. The average of these three values and the standard deviation were determined for each component identified.

GC/MS Analyses. The GC/MS analyses were carried out with an *Agilent 6890N* chromatograph coupled to an *Agilent 5973* MS detector (*Agilent*, F-Massy). Samples were analyzed on a fused-silica cap. column *HP-1* (polydimethylsiloxane, 50 m × 0.20 mm i.d. × film thickness 0.33 µm, *Interchim*, F-Montluçon) and *INNOWAX* (polyethyleneglycol, 50 m × 0.20 mm i.d. × film thickness 0.4 µm; *Interchim*). Carrier gas, He; constant flow, 1 ml/min; injector temp., 250°, split ratio, 1:100; temp. program: 45° to 250° or 230°, at 2°/min, then held isothermal (20 min) at 250° (apolar column) or 230° (polar column), ion source temp., 230°; transfer line temp., 250° (apolar column) or 230° (polar column); ionization energy, 70 eV; electron ionization mass spectra were acquired over the mass range of 35–400 amu. Identification of the components was based on computer matching against commercial libraries (*Wiley*, *MassFinder 2.1 Library*, *NIST98*), laboratory MS libraries built up from pure substances, and MS literature data [38–43], combined with comparison of GC *RI* values on apolar and polar column.

Enzymatic Acylation of Palmarosa Essential Oil. In a 20-ml vial, palmarosa essential oil (50 mg) and the desired acyl donor (1 mmol) were dissolved in toluene (5 ml), and the lyophilized enzyme (*Candida rugosa* lipase, 5 mg) was then added. The vial was capped, and the mixture was incubated at 40° and shaked at 150 rpm until completion. Workup consisted in decantation, withdrawal of the supernatant, toluene removal *in vacuo*, liquid/liquid extraction with Et_2O/aq. 5% Na_2CO_3, collection of the org. phase, drying ($MgSO_4$), filtration, and solvent removal. The reaction progress was estimated by analysis of a parallel reaction performed simultaneously on citronellol and followed by TLC. The final conversion yield was determined by ^{1}H-NMR of the same sample after workup.

Olfactory Evaluation by Trained Perfumers. Samples of pure lipase-modified palmarosa essential oil with three different acyl donors (introducing acetyl, propanoyl, and crotonyl groups) at various conversion yields were submitted on strips to the evaluation of trained perfumers from *Payan Bertrand S.A.*

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