

Discovery of New Lactones in Sweet Cream Butter Oil

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S Supporting Information

ABSTRACT: Sweet cream butter oil was analyzed to identify new volatile compounds that may contribute to its flavor, with an emphasis on lactones. The volatile part of butter oil was obtained by using short-path distillation. As some previously unknown lactones were detected in this first extract, it was fractionated further. The fatty acids were removed, and the extract was fractionated by flash chromatography. Three lactonic fractions possessing a creamy, buttery, and fatty character were investigated in depth by gas chromatography (GC) and mass spectrometry (MS) (EI and CI) and high-resolution GC–time-of-flight MS. Many lactones were identified by their mass fragmentation and by comparison with reference materials synthesized during this work. Six γ -lactones, five δ -lactones, and one ϵ -lactone were identified for the first time in butter oil, seven of them for the first time in a natural product. The possible contribution of these new lactones to the aroma of butter oil is briefly discussed.

KEYWORDS: butter oil, aroma, lactones, short-path distillation, GC–O, GC–MS

INTRODUCTION

Butter aroma has been extensively investigated over the past 60 years.^{1–11} As recently reviewed by Mallia et al.,¹¹ more than 230 volatiles have been identified as natural constituents of butter. However, only a small number of volatile compounds have been recognized as key odorants of butter flavor. Among the mostly cited compounds are diacetyl, butanoic acid, hexanoic acid, hexanal, acetaldehyde, dimethyl sulfide, decan-5-olide, dodecan-5-olide, decanoic acid, phenol, *p*-cresol, and skatole.¹¹

While the aroma of cream results mainly from the aqueous fraction of milk, butter aroma is mostly influenced by the fat fraction.³ Therefore, butter oil obtained after removal of butter-milk by decantation has been extensively investigated.^{12–19} In addition to the abundant lactones previously mentioned, Van der Zijden et al.¹³ reported the occurrence of trace levels of some unsaturated aliphatic lactones, namely, 6-dodecen-4-olide, 9-dodecen-5-olide, and 9-tetradecen-5-olide. Using aroma extract dilution analysis (AEDA), Widder et al.¹⁸ identified diacetyl, decan-5-olide, and (*Z*)-6-dodecen-4-olide as the most potent odorants of butter oil aroma. Lactones are encountered in various ruminant and nonruminant milk fats and result from endogenous animal metabolism.¹⁴ In particular, alkan-4-olides (γ -lactones) and alkan-5-olides (δ -lactones) are considered desirable odorants of butter and butter oil.^{12–16} Six lactones have been intensely studied for their organoleptic contribution to the aroma of butter oil: octan-5-olide, decan-5-olide, dodecan-5-olide, (*Z*)-6-dodecen-5-olide, tetradecan-5-olide, and dodecan-4-olide.^{6,13,16,18} Over the past 30 years, other lactones have been reported for their creamy and dairy notes. Kaiser discovered 4-methyldecan-5-olide, also called *Aerangis* lactone in orchid.²⁰ It has been described as a great enhancer of creamy nuances.²¹ More recently, 4-methyldodecan-5-olide, 4-methyloctan-5-olide, and 4-methyldodecan-5-olide were reported in milk-type flavor compositions²² and in fermented milk flavor compositions.²³ 4-Methylhexan-5-olide was also reported as a flavor-enhancing substance.²⁴ However, none of these methyl-

substituted δ -lactones has been identified in butter. In addition, unsaturated δ -lactones and especially dodecen-5-olide isomers have been patented for their sensory properties.^{25–27} Recently, heavier lactones, namely, tetradecan-4-olide, hexadecan-4-olide, hexadecan-5-olide, and octadecan-5-olide, have been reported as taste-active components of full-fat cream.²⁸ This highlighted again the important role of lactones in dairy products and invited us to study these chemicals in butter oil.

We report here on the analysis of sweet cream butter oil aroma, focusing almost exclusively on lactones. The production of a volatile fraction of butter oil, its fractionation, and the identification and possible aroma contribution of many previously unknown lactones are the subject of this work.

MATERIALS AND METHODS

Materials. Sweet cream butters were purchased in Swiss and French cheese stores. Butter of the brand “Paysan Breton” was produced in Brittany during the summer of 2009.

Standard Products. Unless otherwise specified, commercially available reagents and solvents were purchased from Fluka-Sigma-Aldrich (Buchs, Switzerland), Acros Organics (Geel, Belgium), and SDS Carlo-Erba (Val de Reuil, France). Pentane was freshly distilled before use.

Short-Path Distillation of Butter Oil. The short-path distillation unit of 500 mL was originally purchased from Leybold-Heraeus (Oetikon, Switzerland) more than 30 years ago (product now discontinued by the company). A very similar product is now commercialized by ChemTech (Lockford, IL), model KDL-4.

Butter Paysan Breton (France, 993.6 g) was gently heated in a water bath (40 °C). Melted butter was then centrifuged at 6000 rpm for 20 min at 40 °C to separate the oil phase from the water phase. The recovered

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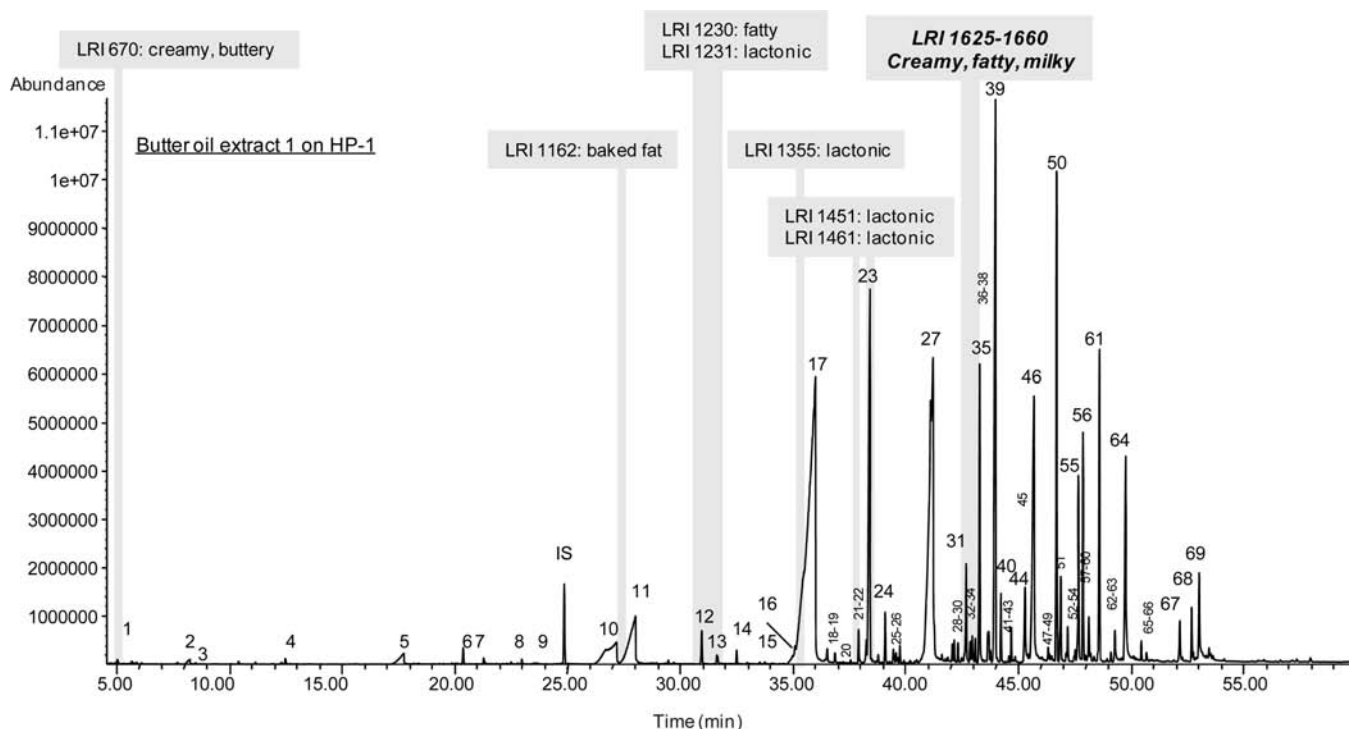


Figure 1. GC–MS trace of BOE-1 (HP-1, 60 m). Butterlike odorant zones obtained by GC–O on BOE-2 (no fatty acids).

butter oil (726.9 g) was introduced in several charges, 100 g per charge at a feed rate of 0.6 mL/min. The short-path distillation unit was set to 50 °C (temperature of the double wall), under 0.09–0.12 mbar, and the rotating part producing the thin film was operated at 350–400 rpm. After each charge, the residues were collected yielding an odorless oil (total of 683.0 g). The nitrogen trap (−195 °C) was rinsed with 3 × 15 mL of freshly distilled pentane and the coldfinger (−20 °C) with 15 mL of freshly distilled pentane. These aroma extracts were combined, dried over Na₂SO₄, and concentrated to 2 mL over a 50 cm Vigreux column to give butter oil extract 1 (BOE-1).

Fatty Acid Removal. Deactivated alumina was obtained by stirring 10 g of alumina with water (0.4 mL) in a rotary evaporator for 2 h. BOE-1 diluted in pentane (10 mL) was deposited onto a Pasteur pipet containing 1.70 g of deactivated alumina. The Pasteur pipet was then rinsed successively with 20 mL of CH₂Cl₂, 5 mL of an 80:20 CH₂Cl₂/diethyl ether mixture, and 5 mL of a 50:50 CH₂Cl₂/diethyl ether mixture. The solvents were combined and concentrated over a 50 cm Vigreux column to 2 mL to give butter oil extract 2 (BOE-2).

Flash Chromatography (FC) over SiO₂. The solid deposition technique was used. BOE-2 was mixed with 1 g of silica gel (32–63 μm, 60 Å), and the solvents were evaporated. The FC column was prepared with 10 g of silica gel in pentane, and the absorbed extract solids were deposited on top of the silica gel bed. Chromatography was performed with a stepwise gradient of pentane and diethyl ether, increasing the percentage of ether as follows: 60 mL (0%), 120 mL (10%), 60 mL (20%), 60 mL (30%), 60 mL (50%), 60 mL (70%), 60 mL (90%), and finally 60 mL (100%). The first 80 mL was discarded, and 160 fractions of 3 mL were collected, which were combined according to their odor evaluation on a smelling strip. The 10 resulting fractions (FC1–FC10) were carefully concentrated over a 50 cm Vigreux column and eventually under a gentle flow of argon and analyzed by GC–MS.

Gas Chromatography–Mass Spectrometry–Olfactometry (GC–MS–O). *Nonpolar Column.* Analyses were conducted using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 MS detector (Agilent Technologies, Palo Alto, CA) and an in-house-built

sniffing port. The column used was an Agilent HP-1 column [60 m × 0.32 mm (inside diameter), 1.0 μm film]. Two microliters of extract was injected using a split injector (250 °C, split ratio of 1:1). The carrier gas was helium with a flow rate of 2.7 mL/min. The temperature program was as follows: 50 °C for 5 min, then gradient of 3 °C/min to 120 °C, then gradient of 5 °C/min to 250 °C followed by a 5 min isotherm, and gradient of 15 °C/min to 300 °C, followed by a 20 min isotherm. The 5973 MS detector was operated in EI (70 eV) or CI mode (NH₃ reagent gas, flow of 30 mL/min in the positive mode and 20 mL/min in the negative mode) and connected to the gas chromatograph with a transfer line heated to 250 °C. Mass spectra were recorded in SCAN mode over the *m/z* 29–350 range. GC sniffing analysis of BOE-2, corresponding to the table given in the Supporting Information, was performed by three experienced judges including one flavorist. GC–FID was performed under the same conditions, with the same instrument except that the column was an Agilent DB-1 MS column [60 m × 0.25 mm (inside diameter), 0.25 μm film] operated at 2.1 mL/min of He.

Polar Column. Analyses were conducted using an Agilent 6890/5973 GC–MS system. The column used was a Supelco (Belleville, PA) Wax column [30 m × 0.25 mm (inside diameter), 0.25 μm film]. One microliter of extract was injected using a split injector (250 °C, split ratio of 1:1). The carrier gas was helium with a flow rate of 0.7 mL/min. For all analyses, the temperature program was as follows: 50 °C for 5 min and then a gradient of 3 °C/min to 240 °C, followed by a 15 min isotherm. The same MS conditions as described above were used in EI mode.

GC–MS peaks were identified and integrated by using HP ChemStation. Linear retention indices (LRIs) were determined after injection of a series of *n*-alkanes (C₅–C₂₈) eluted with the same oven program. The compounds were identified by using MS and LRI libraries (in-house and Wiley) and by comparison with reference samples.

GC–Time-of-Flight (TOF)–MS Analysis. Exact mass measurements were taken using a 7890 gas chromatograph (Agilent) coupled to a GCT Premier time-of-flight mass spectrometer (Waters, Milford, MA). The column used was a Supelco SPB-1 column [30 m × 0.25 mm (inside

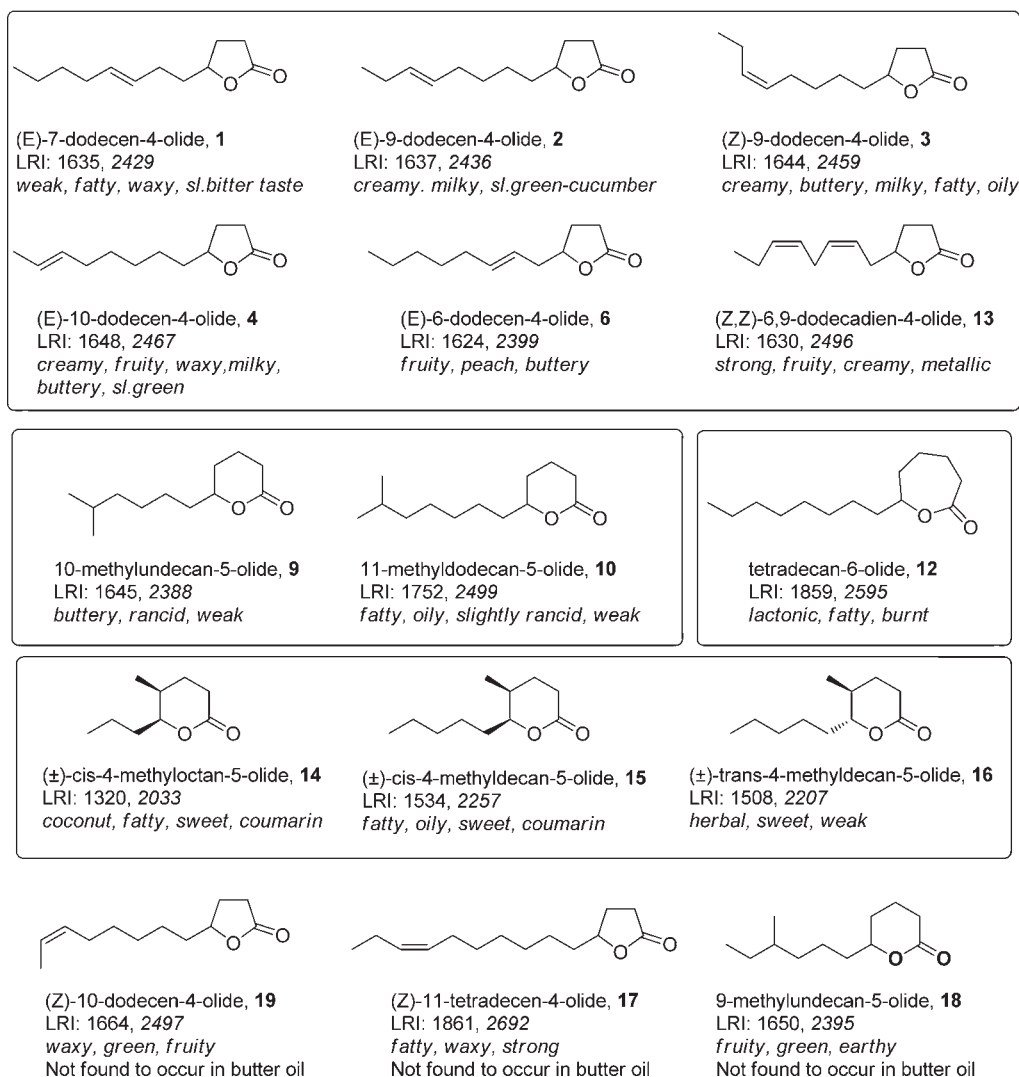


Figure 2. Lactones newly identified in butter oil and lactones synthesized for the purpose of identification but not found in butter oil.

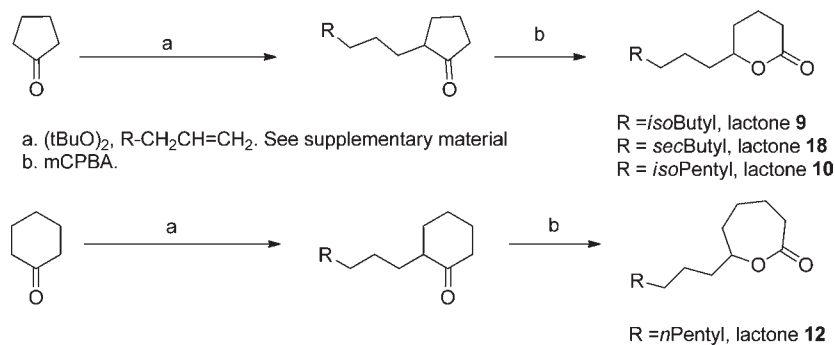


Figure 3. Synthesis of lactones **9**, **10**, **12**, and **18** by Baeyer–Villiger oxidation.

diameter), 0.25 μm film]. One microliter of extract was injected using a split injector (250 $^{\circ}\text{C}$, split ratio of 1:5). The carrier gas was helium with a flow rate of 1.0 mL/min. For all analyses, the oven temperature program was as follows: 60 $^{\circ}\text{C}$ for 5 min and then a 5 $^{\circ}\text{C}/\text{min}$ gradient to 250 $^{\circ}\text{C}$, followed by a 30 min isotherm. The acquisition time was set to 0.49 s with an interscan delay of 0.01 s over a mass range of 1–800 Da. Spectra were recorded by using an electron energy of 70 eV, an emission current of

386 mA, a trap current of 200 mA, and a source temperature of 200 $^{\circ}\text{C}$. Calibration was performed by using heptacosyl (perfluorotributylamine, mass spectrometry grade, Apollo Scientific Ltd., Bradbury, U.K.). Calibration data were collected for 1 min in centroid mode. A total of 60 spectra were summed to generate a 24-point calibration curve from 69 to 614 Da. The curve was fitted to a second-order polynomial such that the standard deviation of the residuals was ≤ 0.001 amu. Heptacosyl was continuously

introduced into the ion source, and the ion at m/z 218.9856 was used as a lock mass. The mass spectra and molecular formula were obtained with MassLynx (Waters).

Synthesis. ^1H and ^{13}C NMR spectra were recorded with a Bruker-Avance 500 MHz spectrometer at 500.13 and 125.76 MHz, respectively. Chemical shifts (δ) and coupling constants (J) are expressed in parts per million and hertz, respectively. The solvent was CDCl_3 with tetramethylsilane as the internal standard. The ^{13}C signal assignments were obtained from standard gradient-selected correlated spectroscopy, heteronuclear single-quantum coherence, and heteronuclear multiple-bond correlation experiments. NMR spectra were processed with Bruker TopSpin 2.0 (s, singlet; d, doublet; t, triplet; m, multiplet).

Synthesis of Lactones. The synthesis of unsaturated γ -lactones, namely, dodecan-4-olides **1–4**, **7**, **13**, and **17** (Figure 2), was recently reported by Frerot and Bagnoud.²⁹ Only the syntheses of lactones **9**, **10**, **12**, and **18** by Baeyer–Villiger oxidation from the corresponding 2-alkylcycloalkanones are described here (Figure 3). The synthesis of the 2-alkylcycloalkanones and their spectral data are provided as Supporting Information.

10-Methylundecan-5-olide, 9. Five grams of 2-(5-methylhexyl)cyclopentanone, 7.44 g of *m*-chloroperbenzoic acid (1.1 equiv, diluted in 20 mL of CH_2Cl_2 and dried over Na_2SO_4), and NaHCO_3 (1 g, 2 equiv) in 60 mL of CH_2Cl_2 were stirred overnight at room temperature. CH_2Cl_2 (150 mL) was added, and the organic phase was washed with 5% sodium sulfite until the peroxide test was negative, dried over Na_2SO_4 , and evaporated. The crude product was distilled over a Vigreux column (bp 91–92 °C, 0.013 mmHg): yield 4.66 g, 86%, 96% by GC–FID; ^1H NMR δ 4.31–4.25 (m, 1H), 2.62–2.55 (m, 1H), 2.49–2.40 (m, 1H), 1.95–1.87 (m, 2H), 1.87–1.80 (m, 1H), 1.76–1.67 (m, 1H), 1.62–1.44 (m, 4H), 1.41–1.26 (m, 3H), 1.20–1.15 (m, 2H), 0.87 (d, $J = 6.7$, 6H); ^{13}C NMR δ 172.0 (s, C(1)), 80.6 (d, C(5)), 38.8 (t, C(9)), 35.9 (t, C(6)), 29.5 (t, C(2)), 27.9 (d, C(10)), 27.8 (t, C(4)), 27.2 (t, C(8)), 25.2 (t, C(7)), 22.6 (q, C(11,11')), 18.5 (t, C(3)); EI-MS m/z (relative intensity) 198 (0.6), 180 (2), 165 (3), 155 (4), 147 (3), 143 (4), 142 (3), 137 (8), 125 (6), 114 (13), 109 (6), 99 (100), 84 (9), 83 (9), 71 (38), 70 (26), 69 (25), 55 (31), 43 (28), 41 (29); HR-ToF-MS 198.1623 ($\text{C}_{12}\text{H}_{22}\text{O}_2$, +1.5 ppm).

11-Methyldodecan-5-olide, 10. The same Baeyer–Villiger protocol was used. From 1.17 g of 2-(6-methylheptyl)cyclopentanone, the crude product was distilled over a Vigreux column (bp 77–78 °C, 0.012 mmHg): yield 0.79 g, 59%, 95% by GC–FID; ^1H NMR δ 4.31–4.25 (m, 1H), 2.62–2.55 (m, 1H), 2.48–2.40 (m, 1H), 1.95–1.87 (m, 2H), 1.87–1.78 (m, 1H), 1.75–1.66 (m, 1H), 1.62–1.46 (m, 4H), 1.41–1.33 (m, 1H), 1.31–1.25 (m, 4H), 1.18–1.12 (m, 2H), 0.86 (d, $J = 6.6$, 6H); ^{13}C NMR δ 172.0 (s, C(1)), 80.6 (d, C(5)), 38.9 (t, C(10)), 35.9 (t, C(6)), 29.7 (t, C(8)), 29.5 (t, C(2)), 27.9 (d, C(11)), 27.8 (t, C(4)), 27.2 (t, C(9)), 25.0 (t, C(7)), 22.6 (q, C(12,12')), 18.5 (t, C(3)); EI-MS m/z (relative intensity) 212 (1), 194 (4), 179 (3), 169 (3), 161 (3), 157 (4), 151 (9), 139 (9), 123 (5), 114 (14), 99 (100), 83 (12), 81 (11), 71 (49), 55 (43), 43 (37), 41 (42); HR-ToF-MS 212.1766 ($\text{C}_{13}\text{H}_{24}\text{O}_2$, –4.7 ppm).

Tetradecen-6-olide, 12. From 2-octylcyclohexanone (5 g), the crude product was distilled over a Vigreux column (bp 89–93 °C, 0.042 mmHg): yield 2.68 g, 50%, 97% by GC–FID; ^1H NMR δ 4.25–4.20 (m, 1H), 2.69–2.57 (m, 2H), 1.96–1.88 (m, 3H), 1.76–1.68 (m, 1H), 1.63–1.57 (m, 3H), 1.55–1.43 (m, 2H), 1.37–1.23 (broad m, 11H), 0.88 (t, $J = 6.7$, 3H); ^{13}C NMR δ 175.8 (s, C(1)), 80.6 (d, C(6)), 36.5 (t, C(7)), 35.0 (t, C(2)), 34.6 (t, C(5)), 31.9 (t, C(12)), 29.5, 29.4, 29.2 (3t, C(9,10,11)), 28.4 (t, C(3)), 25.4 (t, C(8)), 23.1 (t, C(4)), 22.7 (t, C(13)), 14.1 (q, C(14)); EI-MS m/z (relative intensity) 226 (0.2), 208 (1), 190 (1), 164 (3), 151 (1), 141 (2), 128 (3), 124 (4), 113 (35), 98 (8), 97 (8), 96 (6), 95 (9), 85 (100), 84 (69), 69 (23), 67 (29), 57 (20), 56 (40), 55 (78), 43 (25), 41 (42); HR-ToF-MS 226.1933 ($\text{C}_{14}\text{H}_{26}\text{O}_2$, –4.0 ppm).

9-Methylundecan-5-olide, 18. From 4.0 g of 2-(4-methylhexyl)cyclopentanone, the crude product was distilled over a Vigreux column (bp 81–82 °C, 0.015 mmHg): yield 3.91 g, 90%, 96% by GC–FID; ^1H

NMR δ 4.32–4.25 (m, 1H), 2.63–2.55 (m, 1H), 2.49–2.40 (m, 1H), 1.96–1.87 (m, 2H), 1.87–1.78 (m, 1H), 1.75–1.66 (m, 1H), 1.61–1.49 (m, 2H), 1.48–1.40 (m, 1H), 1.37–1.27 (m, 4H), 1.18–1.08 (m, 2H), 0.86 (t, $J = 7.2$, 3H), 0.85 (d, $J = 6.4$, 3H); ^{13}C NMR δ 172.0 (s, C(1)), 80.6 (d, C(5)), 36.3 (t, C(8)), 36.2 (t, C(6)), 34.3 (d, C(9)), 29.5 (t, C(2)), 29.4 (t, C(10)), 27.8 (t, C(4)), 22.5 (t, C(7)), 19.1 (q, $\text{CH}_3\text{-C}(9)$), 18.5 (t, C(3)), 11.4 (q, C(11)); EI-MS m/z (relative intensity) 198 (0.3), 180 (1), 169 (9), 151 (15), 141 (6), 133 (9), 123 (5), 114 (16), 109 (18), 99 (100), 83 (18), 81 (12), 71 (58), 70 (67), 69 (29), 55 (57), 43 (28), 42 (29), 41 (46); HR-ToF-MS 198.1612 ($\text{C}_{12}\text{H}_{22}\text{O}_2$, –4.0 ppm).

Sensory Evaluation of Lactones. Determination of Odor Thresholds by GC–O. An AEDA experiment was conducted to determine the minimal amount of material that must be introduced into the chromatographic system to perceive an olfactory stimulus, as described by Ferreira et al.³⁰ The last solution perceived by one of the two panelists determined the odor threshold, which is given in nanograms (mass perceived by the human nose in the sniffing cone). One microliter of each 0.1, 0.5, 1, 5, or 50 ppm solution of the lactone in ethanol was injected onto the GC–MS–O system with a split ratio of 1:1.

Evaluation of the Lactones in Water. The lactones have been evaluated by a panel of four in-house expert flavorists who tasted these compounds at 20 ppm in water (Figure 2). The descriptor fruity is typically used for decan-4-olide, peach for undecan-4-olide, creamy for tetradecan-5-olide or dodecan-4-olide, buttery for diacetyl, fatty for (*E*, *E*)-2,4-decadienal, oily for oleic acid, waxy for stearic acid, cardboard for (*E*)-2-nonenal, green for (*Z*)-3-hexenol, milky for 5- or 6-decenoic acid, and coconut for nonan-5-olide.

Evaluation of Lactone Blends in Sunflower Oil. Three model solutions containing groups of lactones at 10% (w/w) in sunflower oil were prepared. Solution A consisted of octan-5-olide (15 mg), dodecan-4-olide (111 mg), dodecan-5-olide (358 mg), (*Z*)-6-dodecen-4-olide (36 mg), and tetradecen-5-olide (110 mg) in 5.66 g of sunflower oil. Solution B consisted of **1** (4.1 mg), **2** (5.3 mg), **3** (4.1 mg), and **10** (1.5 mg) in 130.6 mg of sunflower oil. Solution C consisted of **4** (5.0 mg), **6** (4.9 mg), and **13** (4.9 mg) in 136.2 mg of sunflower oil. Then three ready-to-smell solutions X, Y, and Z were prepared as follows. Solution X consisted of 100 mg of solution A and 9.9 g of sunflower oil. Solution Y consisted of 100 mg of solution A, 5 mg of solution B, and 9.9 g of sunflower oil. Solution Z consisted of 100 mg of solution A, 5 mg of solution B, 1 mg of solution C, and 9.9 g of sunflower oil. One milliliter of each solution (X, Y, and Z) was placed in three 70 mL plastic cup. The cups were capped for more than 1 h, and they were evaluated by 10 panelists, including three expert flavorists who were asked to describe the odor and differentiate the samples with regard to the butter oil aroma aspect.

RESULTS AND DISCUSSION

Extraction of Butter Oil Volatiles. In this study, sweet cream butter of the brand Paysan Breton was selected by a panel of flavorists after tasting 20 kinds of French and Swiss butter. It is an industrial butter produced in Brittany, France, and widely distributed in France. When compared to the other brands, it seemed stronger in aroma without any rancid or cardboard note and was appreciated for its milky, buttery, creamy, and fatty notes.

Because of the high fat content of butter oil, the extraction of its odorants is a challenging task. The extent of release of volatiles into the gas phase is significantly decreased with an increasing lipid content of the matrix, and headspace extraction techniques generally lack sensitivity, especially for less volatile odorants.³¹ Several alternative procedures have thus been developed to isolate aroma compounds from fatty matrices, using preferentially high-vacuum systems.^{31,32} We used a special kind of molecular distillation, the short-path distillation, performed on

Table 1. Composition of Butter Oil Extract 1 from GC–MS and GC–FID

	LRI (HP-1)		LRI (Wax)		compound ^a	% FID	% lactone ^b
	in butter	synthesized	in butter	synthesized			
1	675	679	1276	1277	3-hydroxy-2-butanone	0.01	
2	765	767	1599	1606	butyric acid	0.06	
3	773	780	1073	1070	hexanal	0.03	
4	866	866	1174	1172	heptan-2-one	0.09	
5	971	930	2012	1847	hexanoic acid	0.73	
6	1020	1020	1187	1187	limonene	0.24	
7	1037	1052	1778	1807	hexan-5-olide	0.07	0.3
8	1070	1062	1379	1382	nonan-2-one	0.08	
9	1082	1082	1384	1388	nonanal	0.02	
10	1153	1188	>2800	2571	benzoic acid	2.73	
11	1170	1176	2197	2134	octanoic acid	4.05	
12	1236	1237	1953	1974	octan-5-olide	0.39	1.5
13	1253	1251	2427	2440	indole	0.09	
14	1274	1271	1588	1588	undecan-2-one	0.18	
15	1307	1314	2001	1986	<i>cis</i> -4-methyloctan-5-olide	0.03	0.1
16	1346	1348	2474	2491	skatole	2.24	
17	1374	1357	2325	2281	decanoic acid	26.61	
18	1389	1393	1436	1457	1-tetradecene	0.23	
19	1400	1400	1391	1400	tetradecan	0.10	
20	1433	1435	2134	2152	decan-4-olide	0.03	0.1
21	1444		2286		9-decen-5-olide	0.39	1.5
22	1448	1451	not detected	2404	undecanoic acid	0.27	
23	1461	1463	2220	2211	decan-5-olide	7.05	27.3
24	1477	1493	1798	1815	tridecan-2-one	0.49	
25	1496	1493	1737	1727	(<i>Z,E</i>)-farnesene	0.06	
26	1500	1500	1490	1500	pentadecane	0.17	
27	1557	1550	2597	2491	dodecanoic acid	12.75	
28	1590	1585	1636	1624	1-hexadecene	0.16	
29	1594	1591	1910	1921	tetradecanal	0.38	
30	1600	1600	not detected	1600	hexadecane	0.18	
31	1628	1628	2420	2415	(<i>Z</i>)-6-dodecen-4-olide	0.93	3.6
32	1634		2431		Unk. 1	0.21	0.8
33	1637		2441		Unk. 2	0.26	1.0
34	1644		2463		Unk. 3	0.21	0.8
35	1650	1651	2395	2388	dodecan-4-olide	2.88	11.1
36	1667	—	2500	—	(<i>E</i>)-9-dodecen-5-olide	0.27	1.0
37	1671	1672	2511	2507	(<i>Z</i>)-9-dodecen-5-olide	0.27	1.0
38	1655	—	1966	—	unknown	0.10	
39	1681	1680	2450	2443	dodecan-5-olide	9.26	35.8
40	1683	1678	2007	1999	pentadecan-2-one	0.72	
41	1696	—	2015	—	unknown	0.07	
42	1700	1700	1700	1700	heptadecane	0.30	
43	1714	1708	2005	2012	methyl tetradecanoate	0.05	
44	1728	1736	>2800	2745	(<i>Z</i>)-9-tetradecenoic acid	0.91	
45	1736	1752	2527	2499	Unk. 8	0.07	0.3
46	1746	1736	>2800	2699	tetradecanoic acid	4.65	
47	1787	1790	2569	2565	tridecan-5-olide	0.14	0.5
48	1777	1789	2042	2048	ethyl tetradecanoate	0.05	
49	1786	1789	1814	1840	1-octadecene	0.09	
50	1792	1826	1791		1-phytene	4.83	
51	1800	1800	1800		octadecane	0.72	
52	1811				unknown	0.03	

Table 1. Continued

	LRI (HP-1)		LRI (Wax)		compound ^a	% FID	% lactone ^b
	in butter	synthesized	in butter	synthesized			
53	1814				2,6,10,14-tetramethylhexadecane	0.29	
54	1825	1830	2113		6,10,14-trimethyl-2-pentadecanone	0.10	
55	1838		1922		unknown	1.50	
56	1848		1869		(<i>E</i>)-2-phytene	1.75	
57	1853	1843	not detected		tetradecan-4-olide	0.06	0.2
58	1860				unknown	0.46	
59	1864				unknown	0.10	
60	1871				unknown	0.07	
61	1896	1895	2687	2680	tetradecan-5-olide	2.83	10.9
62	1908	1910	2212	2221	methyl hexadecanoate	0.08	
63	1917	1918	not detected		(<i>Z</i>)-9-hexadecenoic acid	0.35	
64	1942	1943			hexadecanoic acid	2.89	
65	1978	1985	2250	2256	ethyl hexadecanoate	0.12	
66	1988		>2800		pentadecan-5-olide (tentative)	0.09	0.3
67	2068		2510		unknown	0.60	
68	2085		>2800		hexadecane-5-olide	0.41	1.6
69	2115		>2800		(<i>Z</i>)-9-octadecenoic acid	1.35	
total						100.00	100.0

^a Identification on the basis of the MS spectra and LRIs. Products that are lacking one LRI should be considered as tentatively identified. ^b Relative proportions of the different lactones.

Table 2. Odor Evaluation and Composition of FC Fractions

fraction	elution ^a	odor quality	composition ^b
FC1	15 (10%)	creamy, green, milky, soapy	alkan-2-ones (2-pentadecanone), aldehydes (hexadecanal), esters (ethyl tetradecanoate)
FC2	18 (10%)	stale, fatty, melted butter	alkan-2-ones (nonan-2-one, heptan-2-one)
FC3	30 (10%)	indolic, floral, honey, skatole	alkan-2-ones (heptan-2-one, 8-nonene-2-one), phenylacetaldehyde, indole, skatole
FC4	30 (10%)	sweet, lauric, burnt, dusty	alcohols, alkan-2-ones
FC5	36 (20%)	weak, animalic, fatty, dusty	phenolic compounds (phenol, <i>p</i> -cresol)
FC6	36 (20%)	weak, melted butter, dusty	fatty alcohols
FC7	48 (30%)	sweet, cream, lactonic, peach	lactones (mainly alkan-4-olides)
FC8	15 (30%)	creamy, butter, cocoa butter, fatty, thick	lactones (mainly alkan-5-olides)
FC9	36 (50%)	coconut, milky, fatty, green	lactones (mainly alkan-5-olides)
FC10	30 (50%)	weak	glycerol monoesters, smaller lactones

^a Elution volume (in milliliters), after the initial 80 mL had been discarded, and solvent composition (percent ether in pentane). ^b As obtained from the GC–MS experiment on the nonpolar column. Typical components are given in parentheses.

the KDL-4 apparatus of Leybold-Heraeus.³³ A detailed picture of this equipment is given in the Supporting Information. In this technique, the oil is introduced dropwise into the high-vacuum chamber where it makes a thin film under the action of Teflon-coated rolling wipers. As the residence time of the product in the distillation chamber at 50 °C is less than a few seconds,³³ the formation of artifacts is greatly minimized. In contrast with SAFE,³² short-path distillation is performed without the assistance of a solvent, thus yielding a more concentrated extract. We thus obtained butter oil extract 1 (BOE-1) that was evaluated by the flavorists as creamy, buttery, and fatty and validated as being representative of the starting butter oil. Figure 1 shows the GC–MS profile of BOE-1, and Table 1 gives its composition as obtained from GC–FID and GC–MS. Free fatty acids accounted for 57.4% of the overall composition, lactones for 25.9%, and ketones for 1.7%. These results were in agreement with previous studies^{2,6,12–16} and showed the importance of

lactones, especially 5-dodecanolide (9.3%), 5-decanolide (7.1%), 5-tetradecanolide (2.8%), and 4-dodecanolide (2.9%). Three unknown products (Unk. 1–3) accounting for 0.2% each and thought to be lactones were already noticed at this stage.

Fractionation of Butter Oil Extract 1. Free fatty acids were removed from BOE-1 by adsorption over aluminum oxide, thus giving butter oil extract 2 (BOE-2), which was evaluated as typical of butter oil although lacking the rancid and sour notes. It was further used for GC–O experiments and fractionation by flash chromatography. GC–O experiments on a nonpolar column were conducted by three experienced panelists, including one flavorist. Many creamy, buttery, fatty odor zones were detected and are shown in Figure 1. The compiled GC–O results together with the tentative identification of the odorants are shown as Supporting Information. BOE-2 was further fractionated by flash chromatography over silica gel using a gradient of pentane and ether designed to achieve a separation by chemical

Table 3. Lactones Identified in Butter Oil

LRI _{apo}		LRI _{pol}		compound	FC fraction	identification ^a	natural occurrence ^b (ref)
in butter	synthesized	in butter	synthesized				
1005	1006	1717	1677	hexan-4-olide	9		butter, ⁴ butter oil ¹⁷
1046	1052	1800	1793	hexan-5-olide	9		butter ⁴
1217	1219	1933	1629	octan-4-olide	8		butter ⁶
1246	1247	1983	1979	octan-5-olide	8		butter ⁴
1297	1297	not detected	1986	<i>trans</i> -4-methyloctan-5-olide	8		not described in food, garden ant ³⁷
1321	1320	2034	2033	<i>cis</i> -4-methyloctan-5-olide 14	9	B	new, synthesized ³⁹
1325	1335	2046	2037	nonan-4-olide	8		butter ¹⁰
1354	1364	2102	2088	nonan-5-olide	9		butter ⁴
1433	1435	2134	2160	decan-4-olide	8		butter, ¹ butter oil ¹⁷
1444	—	2286	—	9-decen-5-olide	8		butter, ⁹ spectral data in ref 27
1461	1463	2220	2211	decan-5-olide	8		butter ⁴
1490	—	2203	—	2,3-dimethyl-2-nonen-4-olide (dihydrobovolide)	7		black tea ⁴¹
1506	1508	2207	2207	<i>trans</i> -4-methyldecan-5-olide 16	8	B	synthesized ³⁸
1530	1534	2257	2257	<i>cis</i> -4-methyldecan-5-olide 15	8	B	orchid ²⁰
1537	1543	2266	2277	undecan-4-olide	8		butter ^{1,2}
1570	1572	2334	2328	undecan-5-olide	8		butter ²
1617	—	not detected	—	Unk. 8 (hypothesized, 3,6-dodecen-4-olide isomer)	8	B	—
1623	1619	2412	2407	Unk. 5 [(<i>Z</i>)-7-dodecen-4-olide (tentative)]	7	B	new
1625	1624	2405	2399	Unk. 6 [(<i>E</i>)-6-dodecen-4-olide]	7	B	new in butter, <i>Morinda citrifolia</i> ⁴⁰
1627	1630	2499	2496	(<i>Z,Z</i>)-6,9-dodecadien-4-olide 13	8	A	milk ^{35,36}
1628	1628	2420	2415	(<i>Z</i>)-6-dodecen-4-olide	7		butter oil ¹⁸
1634	1635	2431	2429	Unk. 1 [(<i>E</i>)-7-dodecen-4-olide]	7, 8	A	new
1637	1637	2441	2436	Unk. 2 [(<i>E</i>)-9-dodecen-4-olide]	8	A	new
1641	1645	2391	2388	Unk. 9 (10-methylundecan-5-olide)	9	B	new, released by <i>Streptomyces</i> species ⁴¹
1644	1644	2463	2459	Unk. 3 [(<i>Z</i>)-9-dodecen-4-olide]	8	A	new
1648	1648	2473	2467	Unk. 4 [(<i>E</i>)-10-dodecen-4-olide]	8	A	new
1650	1651	2395	2388	dodecan-4-olide	8		butter ¹³
1667	—	2500	2495	(<i>E</i>)-9-dodecen-5-olide	8		butter oil ¹³
1671	1672	2511	2507	(<i>Z</i>)-9-dodecen-5-olide	8		butter oil ¹³
1681	1680	2450	2443	dodecan-5-olide	8		butter ¹
1749	1752	2511	2499	Unk. 10 (11-methyldodecan-5-olide)	8	B	new
1758	—	2515	—	tridecan-4-olide	8		butter ¹
1787	1790	2569	2565	tridecan-5-olide	8		butter oil ¹
1849	—	2676	—	Unk. 7 (hypothesized, 10-tetradecen-4-olide)	7	B	—
1856	—	2626	—	Unk. 11 (hypothesized, 11-/ 12-methyltridecan-5-olide)	8	B	—
1860	1843	2630	—	tetradecan-4-olide	7		butter oil ^{1,13}
1860	1859	2595	2595	Unk. 12 (tetradecan-6-olide)	9	B	new
1875	—	2723	—	(<i>Z</i>)-9-tetradecen-5-olide	8		butter oil ¹³
1896	1895	2687	2680	tetradecan-5-olide	8		heated butter, ¹³ butter ¹
1970	—	2744	—	pentadecan-4-olide	8		butter, ¹ butter oil ¹⁷
2000	—	>2800	—	pentadecan-5-olide	8		butter, ¹ butter oil ¹⁷
2077	—	>2800	—	hexadecan-4-olide	7		butter, ¹ butter oil ¹⁷
2110	2113	>2800	—	hexadecan-5-olide	8		butter oil, ¹⁷ cream ²⁸

^a For group A, identification is based on LRI_{apo}, LRI_{pol}, MS (EI and CI modes), and high-resolution ToF MS. For group B, identification is based on LRI_{apo}, LRI_{pol}, and MS (EI and CI modes). Previously known compounds have been identified by injection of reference material, either purchased or from the in-house collection of chemicals that have been fully characterized by NMR. Compounds lacking one LRI should be considered as tentatively identified. ^b New means never identified in a natural product.

functionalities. Table 2 gives the main chemicals found in the 10 fractions, the corresponding elution volumes, the solvent compositions, and the odor descriptions on smelling strips. In particular, fractions FC7–FC9 possessed the creamy, buttery and fatty notes already perceived by GC–O of BOE-2. FC8 was particularly appreciated by the flavorists for its fatty and butter oil odor. This fraction was analyzed by GC–O on a nonpolar column, and the panelists noticed a creamy, fatty, buttery odorant zone between LRI_{apo} values of 1620 and 1660. Interestingly, this zone of the chromatogram presented many previously unknown compounds.

Identification of Lactones in Fractions FC7–FC9 of BOE-2.

The three fractions FC7–FC9 were analyzed by GC–EI-MS on nonpolar and polar columns, by positive and negative GC–CI-MS with ammonia as the reagent gas on a nonpolar column, and by GC–HR-ToF-MS on a nonpolar column. The mass spectra of the unknown compounds as well as those of the corresponding synthetic products are given as Supporting Information. Table 3 lists the lactones identified in these fractions, including many previously unknown compounds. Unknowns 1–6 presented a mass peak at m/z 196 that corresponded to the nominal mass of the molecules as confirmed by positive and negative CI-MS. The presence of an ion at m/z 85 in the electron impact (EI) mass spectra was an indication of the γ -lactone nature of these compounds. High-resolution GC time-of-flight MS indicated C₁₂H₂₀O₂ for the molecular ion. All these spectral data were in accordance with γ -dodecalactones bearing a double bond in the side chain, namely *n*-dodecen-4-olides. The addition of titanium enolate to unsaturated aldehydes was used to prepare many dodecen-4-olides.²⁹ The reference compounds were then used to establish the structure and double bond configuration of the naturally occurring lactones (unknowns 1–6) shown in Figure 2 and Table 3. On the basis of mass spectra and LRI, unknowns 1–3 could be identified as (*E*)-7-dodecen-4-olide 1, (*E*)-9-dodecen-4-olide 2, and (*Z*)-9-dodecen-4-olide 3, respectively. Unknown 4 (LRI_{apo} = 1648; LRI_{pol} = 2473) was identified as (*E*)-10-dodecen-4-olide 4 (LRI_{apo} = 1648; LRI_{pol} = 2467). Its (*Z*)-isomer 19 (LRI_{apo} = 1664; LRI_{pol} = 2497) could not be detected in butter oil. Unknown 5 was tentatively identified as (*Z*)-7-dodecen-4-olide. This compound was obtained as a side product of (*E*)-isomer 1 but was not isolated and fully characterized. Unknown 6 was identified as (*E*)-6-dodecen-4-olide 6.²⁹ The first hypothesis for unknown 7 that presented a nominal mass of 224, namely (*Z*)-11-tetradecen-4-olide 17, turned out to be wrong after comparison with the synthesized material.²⁹ For another hypothesis, (*E*)- or (*Z*)-10-tetradecen-4-olide was not synthesized because of the difficult access of the starting aldehyde to be used with titanium homoenolate.²⁹ The separation provided by flash chromatography allowed identification of (*Z,Z*)-6,9-dodecadien-4-olide 13 in FC8 that was co-eluting with (*Z*)-6-dodecen-4-olide in the whole extract. (*Z,Z*)-6,9-Dodecadien-4-olide 13 was first isolated by Maurer and Hauser from tuberose absolute in 1982.³⁴ More recent studies reported this lactone in skim milk powder³⁵ and in certain cow milk.³⁶ Unknown 8 (Table 3) presented a similar mass spectrum and the same molecular mass of 194 in positive and negative chemical ionization mass spectrometry (CI-MS) with ammonia as the reagent gas. It could not be identified. Three other unknown lactones were found in fractions FC8 and FC9 (unknowns 9–11). CI-MS showed that these lactones had molecular masses of 198, 212, and 226 corresponding to saturated dodeca-, trideca-, and tetrade-lactone, respectively. They could well be methyl-branched

δ -lactones. The presence of an ion at m/z 99, as well the LRIs, which were slightly lower than the corresponding known linear δ -lactones, supported this hypothesis. The occurrence of branched *iso*- and *anteiso*-fatty acids in milk¹⁹ was also an indication of the possible methyl group position. Thus, the two possible products for unknown 9, namely, 9- and 10-methylundecan-5-olide (products 9 and 18, respectively, in Figure 2), were synthesized by using a Baeyer–Villiger reaction (Figure 3). The LRIs were used to identify unknown 9 (LRI_{apo} = 1641; LRI_{pol} = 2391) as 10-methylundecan-5-olide 9 (LRI_{apo} = 1645; LRI_{pol} = 2388), rather than 9-methyldodecan-5-olide 18 (LRI_{apo} = 1650; LRI_{pol} = 2395). Similarly, unknown 10 could be identified as 11-methyldodecan-5-olide 10. We did not synthesize 12-methyltridecan-5-olide, the most likely hypothesis for unknown 11, a trace compound in fraction FC8. In fraction FC9, MS of unknown 12 (LRI_{apo} = 1860; LRI_{pol} = 2595) presented a fragment ion at m/z 113 that denoted a seven-membered ring lactone, whereas its LRI was close to that of tetradecan-5-olide. We thus synthesized tetradecan-6-olide 12 (LRI_{apo} = 1859; LRI_{pol} = 2595), which displayed the same MS fragments as unknown 12. Finally, three other lactones, *cis*-4-methyloctan-5-olide 14³⁷ and *cis*- and *trans*-4-methyldecan-5-olides 15 and 16, respectively,^{38,39} shown in Table 3, had already been described in the literature but had never been shown to occur in butter oil.

Aroma Contribution of the New Lactones. The sensory evaluation of the new lactones tasted by expert flavorists at 20 ppm in water is given in Figure 2. All of them possess odors that are reminiscent of butter oil. The actual contribution of these products to the aroma of butter oil is more difficult to determine. First, the recovery factors for the short-path distillation are unknown. Using thin layer high-vacuum distillation on a model mixture in synthetic oil, Krings et al.³¹ measured recovery factors of many aroma chemicals. For lactones, they obtained 57.4% for octan-4-olide and only 5.1% for dodecan-5-olide. On the other hand, BOE-1 was judged to be representative of the corresponding butter oil. The relative proportions of lactones in BOE-1 are listed in Table 1. They are in line with those found by Jurriens.¹ Among the lactones newly identified in butter oil during this work, the trace compounds that were only detected in flash chromatography fractions are not likely to contribute to the overall aroma. Preliminary GC–O experiments on the nonpolar column have been performed on lactones 1–3 and 6, in comparison with the major lactones [octan-5-olide, decan-5-olide, (*Z*)-6-dodecen-5-olide, dodecan-4-olide, dodecan-5-olide, and tetradecan-5-olide]. The minimal amounts perceived by two panelists were 0.05 ng for dodecan-4-olide; 0.125 ng for lactones 1–3; 0.5 ng for octan-5-olide, decan-5-olide, (*Z*)-6-dodecen-4-olide, and lactone 6; and 25 ng for tetradecan-5-olide. Together with their actual proportions in BOE-1, these results show that lactones 1–3 may contribute to the aroma of butter oil. To evaluate the contribution of the new lactones in oil, we evaluated model mixtures in sunflower oil. Model mixture X consisted of octan-5-olide, decan-5-olide, (*Z*)-6-dodecen-5-olide, dodecan-4-olide, dodecan-5-olide, and tetradecan-5-olide, the major lactones of BOE-1. In addition, model mixture Y contained lactones 1–3 and δ -lactone 10 detected in BOE-1. Finally, in addition, model mixture Z contained lactones 4, 6, and 13. In mixtures X–Z, the lactones were weighed according to their proportions listed in Table 1. Solutions X–Z were perceived as different, showing that minor lactones can still be perceived when added to the major ones. Lactones 1–3 and 10 tended to add a more pronounced creamy and coconut character to mixture Y, while 4, 6, and 13 had an only slight effect on the buttery

character of solution Z. The determination of the odor and taste threshold, as well as their accurate quantification in butter oil, would be necessary to reach a conclusion. A full sensory study of the individual impact of the new lactones is beyond the scope of this work. However, the sensory results presented here provide a clue that the new lactones discovered in this work may contribute to the aroma of butter oil.

■ ASSOCIATED CONTENT

S **Supporting Information.** Additional data and observations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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■ ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; CI, chemical ionization; EI, electron impact; FC, flash chromatography; GC–FID, gas chromatography–flame ionization detection; GC–MS, gas chromatography–mass spectrometry; GC–O, gas chromatography–olfactometry; LRI, linear retention index; SAFE, solvent-assisted flavor extraction; TIC, total ion current; TOF, time-of-flight

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