Stereoselective Enzymatic Synthesis of *cis*-Pellitorine, a Taste Active Alkamide Naturally Occurring in Tarragon

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The alkamide *cis*-pellitorine [(2*E*,4*Z*)-*N*-isobutyldeca-2,4-dienamide] that occurs naturally in tarragon was prepared in yields up to 80% by lipase-catalyzed conversion of ethyl 2*E*,4*Z*-decadienoate, the so-called pear ester, and isobutylamine both with and without the use of cosolvents. Of 13 different commercial enzyme preparations tested (lipases, proteases, esterases), only the lipase type B from *Candida antarctica* has a suitable activity. The reaction of the different geometric isomers of ethyl 2,4-decadienoate to the appropriate pellitorines shows a remarkable selectivity: the 2*E*,4*Z* ester is converted between 1.4 and 3.9 times faster than the 2*E*,4*E* isomer, and the relative yield of *cis*-pellitorine compared with *trans*-pellitorine is 5.7 to 16.3 times higher. In contrast to the better known *trans*-pellitorine, which at 10 ppm is only slightly tingling and numbing, *cis*-pellitorine shows very interesting pungent and warming sensations after tasting trials already in low concentrations of 10 ppm.

Introduction

In contrast to the better known insecticidal pellitorine [(2*E*,4*Z*)-*N*-isobutyldeca-2,4-dienamide, **1a**, Figure 1], which occurs in different plant species such as *Zanthoxylum zanthoxyloides,*[1] *Anacyclum pyrethrum*, and many *Piper ssp.,*[2] the 2*E,*4*Z* isomer ("*cis*-pellitorine", **1b**, Figure 1) was identified in tarragon species (*Artemisia dracunculus*) just recently.[3] Some of the naturally occurring unsaturated alkamides activate afferent neurons directly and are perceived in the mouth and nose mainly by the trigeminal system; typical sensory descriptions for these chemosensates are tingling, pungent and burning.[2] For example, spilanthol [(2*E,*6*Z,*8*E*)-*N*-isobutyldeca-2,6,8-trienamide, **2**] from *Spilanthes acmella* induces saliva flow and a pronounced tingling sensation.[4] In contrast, pellitorine (**1a**) was described as effecting only a numbing sensation on the tongue.[5]

A conventional small scale and multistep synthesis of *cis*-pellitorine was described by Tsukuhara et al.[6] in 24% yield starting from (*E*)-isobutyl-4-acetoxybut-2-enamide using a Wittig sequence with hexanal and subsequent chromatographic workup. The main product in this reaction was the 2*E*,4*E*-isomer **1a**; after characterization, the separated *cis*-pellitorine (**1b**) was isomerized to **1a** using iodine. The coupling method suffers from low stereoselectivity and the use of toxic, air-sensitive, and/or expensive reagents such as selenium dioxide, $Pd(PPh_3)_4$, and butyllithium. It could therefore be very problematical to scale up the described synthesis for commercial purposes.

In our efforts to develop aroma compounds with trigeminal sensations, we have investigated the sensory effects of pellitorine (**1a**) and *cis*-pellitorine (**1b**). Due to the limitations of the conventional organic synthesis, we decided to investigate an enzymatic synthesis of **1b** starting from readily available ethyl 2*E*,4*Z*-decadienoate (**3b**), the so-called pear ester that is widely used for food flavorings and fragrances. This method could be adopted simply to larger

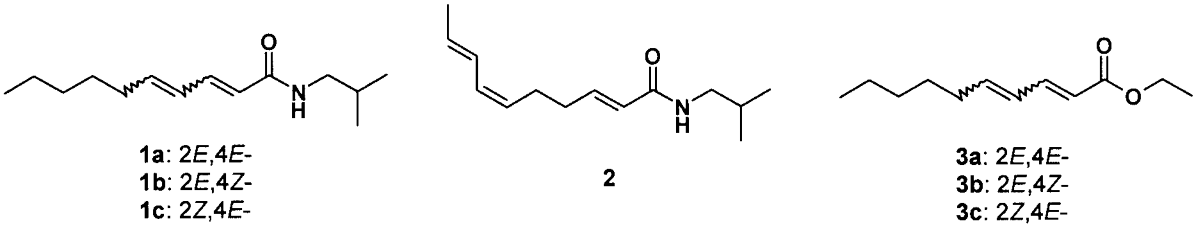

Figure 1. Structures of pellitorines **1**, spilanthol **2**, and ethyl 2,4-decadienoates **3**

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amounts and yields practically usable *cis*-pellitorine. Pellitorine (**1a**) itself could be prepared by the formerly described iodine-catalyzed isomerization of *cis*-pellitorine (**1b**).[6]

Results and Discussion

Various approaches for the enzymatic synthesis of amides of longer chain fatty acids were described earlier. As an example, Liu et al. prepared diethanolamides of fatty acids by direct conversion of the free fatty acids using a lipase from *Candida antarctica* (Novozyme 435) in acetonitrile.[7] In a recently published paper, the enzymatic reaction of medium-chain, saturated carboxylic acids with phenethylamine was performed under reduced pressure using ionic liquids.[8] Some primary amides of saturated short-chain fatty acids and oleic acid were prepared by conversion of mixtures containing ammonia saturated *tert*-butyl alcohol and the appropriate triglyceride using the same enzyme.[9] Several capsaicinoides, for example olvanil (i.e. oleic acid *N*-vanillylamide), were synthesized in a similar manner starting from oleic acid or olive oil and vanillylamine using a lipase, together with hexane as solvent.[10] But for the amidation of medium-chain unsaturated fatty acids, especially α,β-unsaturated, biotechnological methods have not been described so far to the best of our knowledge.

In a first approach we performed a screening to identify an active enzyme using a mixture of 3 diastereomers of ethyl 2,4-decadienoate **3**, which was prepared by iodine-catalyzed partial isomerization of **3b** (the different diastereomers were identical to literature data[11]). We have tested 10 lipases, one esterase, and two proteases, each in three solvents of different polarities. Only the lipase from *Candida antarctica* type B (Chirazyme L2 type, see Table 1) was able to convert the esters **3** to the desired amides **1**. In the most polar solvent, triethylamine, the conversion was higher than in the other solvents. But if the reaction was performed in fivefold-diluted solutions (other conditions unchanged), the conversion of **3b** increased to 48% (heptane), 57% (triethylamine), and 52% (toluene). A remarkable discrimination between the reaction of different diastereomers of ethyl 2,4-decadienoate was seen: the conversion of **3b** was 1.5−12.3 times higher than that of **3c** and 1.4−3.9 times higher than that of **3a**. The yield (GC, from the crude reaction mixture) of 2*E*,4*Z*-pellitorine isomer **1b** relative to the 2*Z*,4*E* (**1c**) and 2*E*,4*E* (**1a**) isomers was 2.6−12.6 and 5.7−16.3 times higher, respectively. The ratios of yields for **1b**/**1a** were lowest for the most polar solvent, triethylamine. As a result, the main product in the enzymatic reaction was *cis*-pellitorine (**1b**), regardless of the composition of the starting ester mixture. These results suggest that in the active site of the lipase, the alkyl chain of the acid prefers a bent conformation at C4−C5.

Thus, for the development of a practical synthesis of *cis*-pellitorine (**1b**), we used Chirazyme L2, c.f. C2 lyo preparation, a commercially available lipase type B from *Candida antartica*, which is supported on a carrier. First, a medium-sized reaction (50 mmol) starting from nearly pure ethyl 2*E*,4*Z*-decadienoate (86% **3b**, 6.2% **3a**, some minor isomers), without any cosolvent, was carried out. The solvent was not necessary for the reaction. The reaction time was lengthened to 4 days, and the amount of enzyme preparation doubled. After dilution and filtration, the crude reaction mixture was treated with KOH to saponify the remaining esters **3** and purified by flash chromatography to yield 80% of **1b** (purity >99% by GC). *cis*-Pellitorine (**1b**) was characterized by GC-MS, HRMS, ^{1}H NMR, and ^{13}C NMR spectroscopic techniques. The coupling constant of H^4 and H^5 of the decadienoic acid part of **1b** is 10.8 Hz and therefore it is clearly *Z*-configured (see Table 2). The data are identical to the reported data for **1b** isolated from tarragon.[3] For comparison, pellitorine (**1a**) was synthesized by iodine-catalyzed isomerization of the *cis*-isomer **1b** according to the literature; the analytical data are identical

Table 1. Screening of different enzymes: 0.25 mmol ethyl 2,4-decadienoates **3**, 0.20 mmol isobutylamine, 10 mg enzyme, 1 mL solvent, 24 h at 50 °C; the relative yields of isomers **1** is referred to the starting amount of the ester isomers **3**; only active enzymes are shown; esterase E 3019 (Sigma), pancreatic lipase (Biocatalysts), lipase F-EC (Extrakt Chemie), lipase PS (Amano), Chirazyme L-8 Lyo (Roche), Chirazyme L-9, c.-f., C2 Lyo (B) (Roche), Chirazyme L-9, c.-f., C2 Lyo (A) (Roche), lipase from *Pseudomonas fluorescens* (Fluka and Biocatalysis), Flavourzyme 500 L (Novo), and Papain P 3375 (Novo) showed no conversion

		GC-%						Conversion (%)			Relative yield (%)			Ratio of conversion		Ratio of yields	
Enzyme	Solvent	**3c**	**3b**	**3a**	**1c**	**1b**	**1a**	**3c**	**3b**	**3a**	**1c**	**1b**	**1a**	**3b/3c**	**3b/3a**	**1b/1c**	**1b/1a**
Starting material		17.9	30.0	46.7	0	0	0										
Chirazyme L-2 C1	heptane	15.4	23.7	39.9	0.1	2.1	0.2	14	21	15	0.6	7.0	0.4	1.5	1.4	12.5	16.3
	triethylamine	16.7	19.3	41.0	1.0	10.6	2.9	7	36	12	5.6	35.3	6.2	5.3	2.9	6.3	5.7
	toluene	17.6	23.8	43.6	0.9	5.2	0.5	2	21	7	5.0	17.3	1.1	12.3	3.1	3.4	16.2
Chirazyme L-2 C2	heptane	15.5	23.0	40.0	0.6	2.7	0.3	13	23	14	3.4	9.0	0.6	1.7	1.6	2.7	14.0
	triethylamine	16.5	16.6	41.3	1.0	14.0	2.6	8	45	12	5.6	46.7	5.6	5.7	3.9	8.4	8.4
	toluene	17.4	25.2	43.8	0.9	3.9	0.4	3	16	6	5.0	13.0	0.9	5.7	2.6	2.6	15.2
Chirazyme L-2 C1[a]	heptane	15.5	15.5	39.6	1.0	12.2	1.3	13	48	15	5.6	40.7	2.8	3.6	3.2	7.3	14.6
	triethylamine	15.9	12.8	28.3	0	13.6	3.1	11	57	39	0.0	45.3	6.6	5.1	1.5	–	6.8
	toluene	15.6	14.4	39.2	1.2	13.6	1.3	13	52	16	6.7	45.3	2.8	4.0	3.2	6.8	16.3

5 mL solvent.

Table 2. Configuration of the diene moiety of the different isomers of pellitorine **1a**−**1c**; only the relevant ^{1}H NMR spectroscopic data are presented (400 MHz, CDCl$_3$, TMS as internal standard)

Compound	RI/DB 1	RI/WAX	δ_{H2}/ppm	δ_{H3}/ppm	$^3J_{H2H3}$/Hz	δ_{H4}/ppm	δ_{H5}/ppm	$^3J_{H3H4}$/Hz	$^3J_{H4H5}$/Hz
1b[a]	1848	2719	5.82	7.56	14.9 (*E*)	6.08	5.79	11.5	10.8 (*Z*)
1a[a]	1888	2803	5.75	7.19	14.9 (*E*)	6.13	6.07	9.7	15.1 (*E*)
1c[b]	1790	2613	5.46	6.38	11.2 (*Z*)	7.45	5.97	11.2	15.3 (*E*)

[a] Signals are correlated by HECTOR experiments. [b] Signals are correlated by a TOCSY experiment.

to that reported earlier.[6] The ^{1}H NMR spectroscopic data of **1a** shows high coupling constants of the protons on both double bonds, which is typical for the *E* configuration (Table 2). The third investigated isomer **1c** of pellitorine was only isolated from a crude product of the enzyme-catalyzed conversion. For **1c**, the coupling constants of the protons located on the double bonds in the ^{1}H NMR spectrum strongly suggest the 2*Z*,4*E* configuration (Table 2).

For the scaling up of the reaction, the reaction was best performed in toluene at 50 °C, by adding the amine in 5 to 6 portions over the reaction time. The reaction also proceeds very well in *tert*-butyl methyl ether or without any cosolvent. It was difficult to stir the solvent-free variant since the mixture had a high viscosity. Triethylamine was not used due to its intense smell, which would require an additional acidic purification step, and due to the lower selectivity. Conversion of **3** was mostly complete (all isomers $<5\%$) mainly after 100−150 h and was independent of the reaction volume. In Figure 2, the conversion of **3a** and **3b** to the main products **1a** and **1b** is plotted against reaction time. Compound **3b** was mostly converted into **1b** after 100 h. Compound **1a** formed much slower than isomer **1b**. Only a small amount of the ester **3a** was converted during the reaction time.

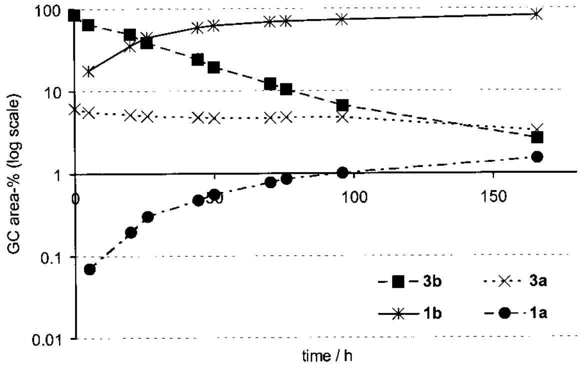

Figure 2. Plot of the reaction of ethyl 2,4-decadienoates **3** with isobutylamine using Chirazyme L2, c.f., C2, lyo to form pellitorines **1** in toluene at 50 °C against time; isobutylamine is added in six equal-sized portions during the reaction period

When the amine was added in one portion, a side reaction occurs, which proceeds faster than the amidation. As can be seen in Figure 3, the slope of the formation of **4** is much higher than that for pellitorine **1a**, especially in the early phase of the reaction. In order to decrease the amount of **4**, the actual excess isobutylamine should be diminished during conversion and the reaction time should not be lengthened. If the reaction mixture was allowed to stir for a longer time (>100 h), the side product **4** is formed in higher amounts (up to 24% GC area) and can be identified by HPLC/ NMR analysis of the crude residue. The molecular mass of 298 determined by GC/MS indicates that nucleophilic addition of isobutylamine occurs to form **4**. The ^{1}H NMR spectrum displays the typical pattern for the *N*-isobutylalkylamide group. One disubstituted double bond was defined by an ABX spin system at 5.35 and an ABX$_2$ spin system at 5.86 ppm. The *E*-configuration was confirmed by a coupling constant of $^3J = 15.3$ Hz between H-3 and H-4. The position of the double bond (C-3/C-4) was assigned by cross coupling of the proton at C-3 with the methylene protons (d, 2.92 ppm) at C-2 in the WET-DQF-COSY spectrum. The proton at C-4 showed cross coupling with the methine proton (ddd, 3.45 ppm) at C-5, which further coupled with one of the methylene protons (m, 1.49 ppm) at C-6. The results can be explained by nucleophilic attack of isobutylamine at C-5 to form the aza-Michael adduct **4** of pellitorines **1** (see Figure 4). Normally the aza-Michael reaction requires strong bases or Lewis acids to activate either the nucleophile or the acceptor. In a control reaction that involves the heating of ethyl 2,4-decadienoates **3** and excess isobutylamine without any enzyme, no conversion of the esters occurs. In addition, the Michael product **4** is not detected and pellitorines are not formed under these conditions. Thus, it may be possible that during the enzymatic synthesis of **1** the Michael reaction was also catalyzed by the lipase. This type of enzymatic reaction is only very ra-

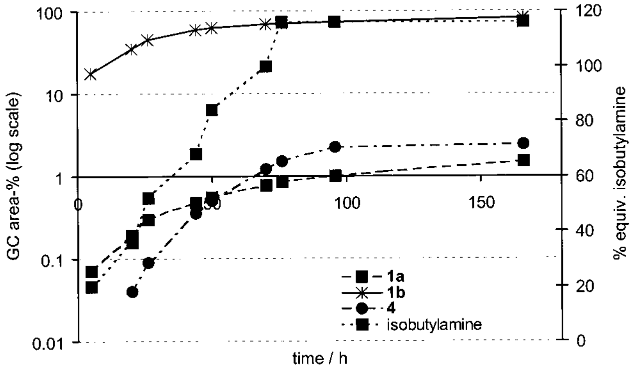

Figure 3. Formation rate of the pellitorines **1** relative to the side reaction leading to **4**: same conditions as those used for Figure 2

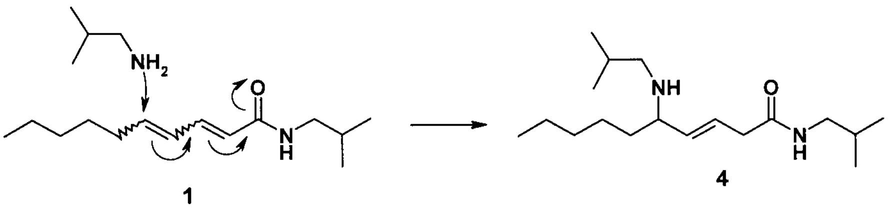

Figure 4. Proposed formation of side product **4** by a Michael reaction of **1** with isobutylamine

Table 3. Sensory profile of pellitorines **1a** and **1b** relative to that of dihydrocapsaicine (6−8 test persons, trained panel)

Test compound	Concentration in 11% aqueous sucrose solution	Sensory description
cis-Pellitorine (**1b**)	10 ppm	distinct warm feeling, immediately pungent, typical ethanol pungency
Dihydrocapsaicine	100 ppb	delayed action mainly at throat, burning, aggressive, no warm feeling
Pellitorine (**1**a)	10 ppm	mouthwatering, fatty, fruity, slightly tingling, numbing, not pungent

rely described in literature; for example Cai et al. reported a Michael addition of pyrimidine derivatives to 2,3-unsaturated alkyl alkenoates using an alkaline protease from *Bacillus subtilis*.[12] Further studies on this reaction will be done.

The maximum yield of **1b** in crude product mixtures was about 80% (GC); the by-products were mainly unchanged 2,4-decadienoic acid esters **3**, pellitorine (**1a**), and pellitorine isomer **1c**. Because of the side reaction that yields **4**, the next portion of isobutylamine should not be added prior to full conversion of the amine, especially in the case of larger batches. Using this protocol, **4** is only produced in amounts less than 2% (GC area of crude reaction mixture), which can be reduced to <0.1% by simple filtration through silica gel using ethyl acetate/hexane as solvent. It was advantageous to add fresh enzyme if the conversion of ester was too low. In one trial, the reaction stopped at a concentration of 73% of **1b** (GC area) after 96 h (69% after 70 h); by adding fresh enzyme the yield increased to 83% (GC area) after stirring for a further 72 h. The enzyme can be used again after thoroughly washing it with toluene.

For sensory studies, small quantities of **1a** and **1b** were purified again by flash chromatography; in contrast to the 2*E*,4*E* isomer pellitorine (**1a**), *cis*-pellitorine (**1b**) showed a profound pungent and warming sensation (see Table 3). The capsaicinoid dihydrocapsaicin was used as a reference for pungent compounds. According to the literature, the 2*E*-double bond in fatty acid alkamides is necessary for activity, but without further unsaturation in the alkyl chain they show no strong sensation, as exemplified for (2*E*)-*N*-isobutyldec-2-enamide and (2*E*)-*N*-isobutyltetradec-2-enamide.[13] Thus, the stereochemistry of the double bond at C4/C5 shows an important influence on the sensory quality and intensity. This effect has never been described in sensory evaluations to date and is a new aspect of the known structure/activity relationships for the sensation of various alkamides.[13,14]

Conclusion

The synthesis of *cis*-pellitorine (**1b**) starting from pear ester (**3b**) and isobutylamine, and catalyzed by a commercial preparation of *Candida antarctica* lipase type B, yielded 80% product (GC crude product), which could be further purified by chromatography (99% purity, 80% isolated yield). Alkamide **1b**, which occurs in tarragon, showed very interesting pungent and warming sensations and therefore may be used as an aroma compound, for example for spicy food applications.

Experimental Section

General Remarks: Pancreatic lipase was from Biocatalysts (Wales, UK), lipase PS from Amano (Nagoya, Japan), lipase F-EC from Extrakt Chemie (Stadthagen, Germany), Chirazyme L-2 C1, Chirazyme L-2 C2, Chirazyme L-8 lyo., Chirazyme L-9, c.-f., C2 Lyo (B), Chirazyme L-9, and Chirazyme c.-f., C2 Lyo (A) were from Roche Diagnostics (Basel, Switzerland), Flavourzyme 500 L was from Novozymes A/S (Bagsvaerd, Denmark), esterase E 3019, papain P 3375, lipase from *Pseudomonas fluorescens* 62312 from Sigma (Deisenhofen, Germany). D_2O (99.9%) was from Deutero GmbH (Kastellaun, Germany), acetonitrile (HPLC/ NMR grade) from Riedel de Haën (Seelze, Germany), other solvents were from E. Merck (Darmstadt, Germany) or Acros Organics (Geel, Belgium), and all other chemicals were from Sigma−Aldrich (Deisenhofen, Germany), Lancaster Synthesis (Frankfurt, Germany), or Acros Organics (Geel, Belgium). Preparative flash chromatography was performed using the Flash 40 system (Biotage, USA).
NMR spectra were recorded on Varian Gemini2000 (200 MHz) or Varian ^{Unity}INOVA (400 MHz) spectrometer. LC/NMR experiments were performed on the ^{Unity}INOVA system using a $^{1}H\{^{13}C/^{15}N\}$PFG triple resonance indirect detection microflow LC/NMR probe (IFC probe) with a detection volume of 60 μL at 20 °C. The HPLC system consisted of a ternary Varian ProStar 230 pump, a Varian ProStar 330 Photodiode Array Detector (210 nm) and a

Varian ProStar 510 column oven (50 °C). Chromatographic separation was carried out on an OmniSpher C18 column (250 × 4.6 mm, particle size 5 µm) with gradients with acetonitrile and D_2O (contains 0.01% TFA v/v). Solvent suppression was employed by executing WET pulse sequence before every acquisition.

For preparative gas chromatography, a Gerstel MCS-system HP5980 Series gas chromatograph equipped with a KAS 3 injector and a flame ionization detector (300 °C) was used. Preparative separations were performed on a wide bore DB-WAX fused silica column (30 m × 0.53 mm i.d./ d_f = 1.0 µm). The column temperature was programmed from 80 °C to 230 °C at 4 °C/min. Helium carrier gas was used with a column flow of 5 mL/min.

High resolution mass spectra were recorded using a Finnigan MAT 8200 (Finnigan MAT, Bremen, Germany) by flash evaporation of samples after dissolving in methanol (resolution > 8000). GC/EI-MS were recorded on Finnigan SSQ 7000 (Finnigan MAT Bremen) at 70 eV ionization energy, 220 °C ion source temperature applying DB1 fused silica column (30 m, 0.25 mm i.d., 0.25 µm film).

Kovacs Indices (retention index, RI) have been determined by GC on DB1 (20 m, 0.18 mm i.d., 0.18 µm film) and the GC oven temperature was programmed from 60 °C to 300 °C at 12 °C/min. Hydrogen carrier gas was used with a constant flow of approximately 1 mL/min.

Mixture of Different Diastereoisomers of Ethyl 2,4-Decadienoate (3): Commercial ethyl (2*E*,4*Z*)-deca-2,4-dienoate (**3b**, 200 g) was isomerized in toluene (650 mL) by treatment with iodine (2.5 g). The resulting mixture was stirred for 48 h at room temperature, washed with saturated $Na_2S_2O_3$ solution (two 200 mL portions) and water (200 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated, and the residue fractionated in vacuo. A fraction composed of 2*E*,4*Z* isomer **3b** (30%, RI 1443), 2*E*,4*E* isomer **3a** (47%, RI 1483) and 2*Z*,4*E* isomer **3c** (18%, RI 1429) was used for the following enzyme screening.

Screening of Enzymes: The ethyl 2,4-decadienoate mixture (54 µL, 48.7 mg, 0.25 mmol) and isobutylamine (20 µL, 14.7 mg, 0.20 mmol) were mixed in a polyethylene vessel with an appropriate solvent (heptane, triethylamine, toluene, 1 mL). Sodium sulfate (10 mg) and the enzyme (10 mg) were added, and the mixtures shaken at 50 °C for 24 h. The mixtures were centrifuged, the supernatant decanted and analyzed using GC. For further evaluation, the same reaction was performed in 5 mL solvent using the amounts of substrates and enzyme described earlier.

(2*E*,4*Z*)-*N*-Isobutyldeca-2,4-dienamide (1b): Ethyl (2*E*,4*Z*)-deca-2,4-dienoate (**3b**) (10 g, 50.9 mmol), Chirazyme L-2 (c.f., C2, lyo., 4.7 g, activity of carrier fixed type 200 u g^{-1} in contrast to 120 u mg^{-1} for the simple protein[15]), and isobutylamine (4 g, 54.7 mmol) were stirred at 55 °C for 4 days. The mixture was diluted by diethyl ether (100 mL) and filtered; the filtrate was evaporated to dryness in vacuo (yield of raw material 15.2 g). The product was stirred in 10% KOH/methanol (1:1 w/w) at room temperature for 45 min and extracted with diethyl ether. The organic phase was dried over Na_2SO_4, filtered, and the filtrate was evaporated to dryness in vacuo. The saponified raw material was chromatographed on silica gel 60 using hexane/ethyl acetate 10:1 (v/v) as eluent. Yield 9.1 g (40.7 mmol, 80%, GC: RI 1844, 99.4%). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.88 [t, J = 6.5 Hz, 3 H, C(10)−H], 0.93 [d, J = 6.7 Hz, 6 H, C(3′)-H + C(4′)−H], 1.50−1.22 [m, 6 H, C(7)−H, C(8)-H, C(9)−H], 1.81 [m, J = 6.7 Hz, 1 H, C(2′)−H], 2.22−2.36 [m, 2 H, C(6)−H], 3.18 [dd, J = 6.8, J = 6.1 Hz, 2 H, C(1′)−H], 5.50 (bs, 1 H, N−H), 5.79 [dtdd, J = 10.8, J = 7.8, J = 0.9, J = 0.9 Hz, 1 H, C(5)−H], 5.82 [d, J = 14.9 Hz, 1 H, C(2)−H], 6.08 [ddtd, J = 11.5, J = 10.8, J = 1.4, J = 0.6 Hz, 1 H, C(4)−H], 7.56 [ddd, J = 14.9, J = 11.5, J = 1.0 Hz, 1 H, C(3)−H] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 14.02 [CH_3, C(10)], 20.15 [2 × CH_3, C(4′) + C(3′)], 22.52 [CH_2, C(9)], 28.15 [CH_2, C(6)], 28.63 [CH, C(2′)], 29.14 [CH_2, C(7) or C(8)], 31.41 [CH_2, C(7) or C(8)], 46.96 [CH_2, C(1′)], 123.78 [CH, C(2)], 126.28 [CH, C(4)], 135.76 [CH, C(3)], 140.07 [CH, C(5)], 166.34 [C, C(1)] ppm, MS (GC/EI MS): *m*/*z* = 29 (9%), 41 (16%), 53 (12%), 57 (12%), 67 (18%), 69 (14%), 81 (49%), 96 (33%), 110 (12%), 113 (17%), 138 (5%), 151 (100%, base peak), 166 (7%), 180 (10%), 208 (10%), 223 (29, $M^{+\bullet}$), HRMS: M^+ calcd. for $C_{14}H_{25}NO$, 223.1936; found, 223.1938.

(2*E*,4*E*)-*N*-Isobutyldeca-2,4-dienamide (1a): (2*E*,4*Z*)-*N*-Isobutyldeca-2,4-dienamide (15 g) (**1b**) was treated by iodine (2.13 g) in toluene (50 mL) at room temperature for 30 h. The solvents were evaporated in vacuo, and the oily residue recrystallized from petroleum ether (b.p. 60−95 °C). Yield 3.5 g (23%. GC: RI 1889, 94.9%, RI 1848 3.7%, RI 1787 0.1%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.89 [t, J = 6.9 Hz, 3 H, C(10)−H], 0.93 [d, J = 6.7 Hz, 6 H, C(4′)−H + C(3′)−H], 1.37−1.22 [m, 4 H, C(8)-H, C(9)−H], 1.42 [m, J = 7.1 Hz, 2 H, C(7)−H], 1.80 [m, J = 6.7 Hz, 1 H, C(2′)−H], 2.14 [dt, J = 7.0, J = 6.4 Hz, 2 H, C(6)−H], 3.17 [dd, J = 6.9, J = 6.1 Hz, 2 H, C(1′)−H], 5.50 (bs, 1 H, N−H), 5.75 [d, J = 14.9 Hz, 1 H, C(2)−H], 6.07 [dt, J = 15.1, J = 6.4 Hz, 1 H, C(5)−H], 6.13 [dd, J = 15.1, J = 9.6 Hz, 1 H, C(4)−H], 7.19 [m, J = 14.9, J = 9.7 Hz, 1 H, C(3)−H] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.02 [CH_3, C(10)], 20.13 [2 × CH_3, C(4′) + C(3′)], 22.48 [CH_2, C(9)], 28.49 [CH_2, C(7)], 28.64 [CH, C(2′)], 31.38 [CH_2, C(8)], 32.93 [CH_2, C(6)], 46.83 [CH_2, C(1′)], 121.74 [CH, C(2)], 128.21 [CH, C(4)], 141.32 [CH, C(3)], 143.23 [CH, C(5)], 166.41 [C, C(1)] ppm, MS (GC/MS EI): *m*/*z* = 29 (14%), 41 (26%), 53 (20%), 55 (18%), 57 (15%), 66 (16%), 67 (23%), 69 (20%), 81 (67%), 96 (65%), 110 (16%), 113 (19%), 151 (100%, base peak), 166 (8%), 180 (8%), 208 (10%), 223 (34, $M^{+\bullet}$).

(2*Z*,4*E*)-*N*-Isobutyldeca-2,4-dienamide (1c): An enriched fraction of diastereomer **1c** (50%, 25% **1a** and minor components) was obtained by preparative GC of a pellitorine crop and analyzed by NMR spectroscopy and GC/MS. ^{1}H NMR (400 MHz, $CDCl_3$, only peaks for the 2*Z*,4*E* isomer **1c**, correlated by a TOCSY experiment): δ = 0.9 [m, 3 H, C(10)−H], 0.94 [d, J = 6.7 Hz, 6 H, C(4′)−H + C(3′)−H], 1.44−1.20 [m, C(7)-H, C(8)−H, C(9)−H], 1.81 [m, J = 6.8 Hz, C(2′)−H], 2.00−2.24 [m, C(6)−H], 3.14 [dd, J = 6.8, J = 6.1 Hz, 2 H, C(1′)−H], 5.46 [m, J = 11.3 Hz, 1 H, C(2)−H], 5.52−5.40 [bs, 1 H, N−H], 5.97 [m, J = 15.3, J = 7.0, J = 0.8 Hz, 1 H, C(5)−H], 6.38 [ddd, J = 11.7, J = 11.2, J = 0.8 Hz, 1 H, C(3)−H], 7.45 [dddt, J = 15.3, J = 11.2, J = 1.5, J = 1.0 Hz, 1 H, C(4)−H] ppm. MS (GC/MS EI): *m*/*z* = 41 (7%), 67 (7%), 81 (17%), 96 (23%), 152 (100%, base peak), 166 (5%), 180 (5%), 194 (2%), 208 (2%), 223 (13%, $M^{+\bullet}$).

***N*-Isobutyl-4-(isobutylamino)dec-3-enamide (4):** Ethyl (2*E*,4*Z*)-deca-2,4-dienoate (**3b**, 20 g, 102 mmol), isobutylamine (8 g, 110 mmol) and Chirazyme L-2 (c.f., C2, lyo., 4.7 g) were stirred at 55 °C for 7 days. The crude reaction mixture was diluted with diethyl ether (100 mL), filtered, and the filtrate evaporated to dryness to yield a crude product (15.1 g) containing **3b** (4%), **1b** (71%), **1a** (2.5%), and **4** (7.4%). The side product **4** was analyzed by GC/MS (RI DB 1 2088) and HPLC/ NMR spectroscopy starting from the crude batch. LC separation was performed with a gradient with D_2O (containing 0.01 TFA% v/v) and acetonitrile (95:5 − 30 min − 5:95

−15 min − 5:95) in stop-flow mode (1 mL/min). Signals that could be certainly assigned by ^{1}H, DQF-COSY and gHSQC experiments: ^{1}H NMR (400 MHz, CH$_3$CN/D$_2$O): δ = 0.76 [t, J = 6.6 Hz, 3 H, C(10)−H], 0.76 [d, J = 6.7 Hz, 6 H, C(3′)−H], 0.83 [d, J = 6.7 Hz, 6 H, C(3′′)−H], 1.08−1.22 [m, 6 H, C(7)-H − C(9)−H], 1.43−1.55 [m, 1 H, C(6)−H$_a$], 1.64 [ddsept., J = 6.4, J = 6.4, J = 6.4 Hz, C(2′)−H], 1.61−1.72 [m, 1 H, C(6)−H$_b$], 2.61 [dd, J = 12.4, J = 6.8 Hz, 1 H, C(1′′)−H$_a$], 2.66 [dd, J = 12.4, J = 7.5 Hz, 1 H, C(1′′)−H$_b$], 2.86 [d, J = 6.8 Hz, 2 H, C(1′)−H], 2.92 [d, J = 7.1 Hz, 2 H, C(2)−H], 3.45 [ddd, J = 10.3, J = 10.3, J = 3.4 Hz, 1 H, C(5)−H], 5.35 [dd, J = 15.3, J = 9.5 Hz, 1 H, C(4)−H], 5.86 [ddd, J = 15.4, J = 7.1, J = 7.1 Hz, 1 H, C(3)−H] ppm. ^{13}C NMR (100 MHz, CH$_3$CN/D$_2$O): δ = 13.9 [CH$_3$, C(10)], 19.1 [4 × CH$_3$, C(3′), C(3′′)], 22.0 [CH$_2$, C(9)], 24.4 [CH$_2$, C(7)], 30.3 [CH$_2$, C(8)], 30.8 [CH$_2$, C(6)], 39.5 [CH$_2$, C(2)], 46.8 [CH$_2$, C(1′)], 52.2 [CH$_2$, C(1′′)], 61.4 [CH$_2$, C(6)] ppm, MS (GC/MS EI): m/z = 41 (57%), 57 (81%), 67 (31%), 81 (31%), 96 (66%), 110 (17%), 126 (51%), 152 (93%), 168 (7%), 182 (15%), 196 (13%), 207 (6%), 225 (100%, base peak), 253 (11%, $M^{+\bullet} - C_3H_7$). LC/MS (ESI MS): m/z = 297 $[M + H]^+$.

Scale Up of (2*E*,4*Z*)-*N*-Isobutyldeca-2,4-dienamide (1b): To ethyl (2*E*,4*Z*)-deca-2,4-dienoate (100 g, 0.509 mol) (**3b**) and Chirazyme L-2 (20 g) in toluene (50 mL), isobutylamine (40 g, 0.547 mol) was added in six equal-sized portions over 2 days (0 h, 5 h, 19 h, 26 h, 40 h, 48 h). After 40 h, a further 20 g of Chirazyme L-2 were added. After 80 h, the mixture was filtered through silica gel G60, and the filtrate was evaporated at 40 °C/80 mbar to yield a technical product (90.7 g) containing 81% *cis*-pellitorine (**1b**), 1.6% pellitorine (**1a**), and ethyl deca-2,4-dienoates **3b** (5.3%), **3a** (6.7%) and side product **4** (< 0.1%).

Sensory Studies: The test compound was dissolved in ethanol (10 mg/5 mL for pellitorines **1a** and **1b**, 1 mg/50 mL for dihydrocapsaicin) and 1 mL of the solution further diluted with 11% sucrose in water (200 mL). For evaluation, the test solutions (2−5 mL) were sipped for 10−20 seconds and than spat out. After each test the mouth chamber was rinsed using 11% aqueous sucrose solution. Tasting sessions were performed by six to eight fully informed and trained panelists.

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