Tetrahedron: *Asymmetry* 24 (2013) 1207–1211

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

A concise and stereoselective chemoenzymatic synthesis of Sitophilate, the male-produced aggregation pheromone of *Sitophilus granarius* (L.)

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ARTICLE INFO

Article history:
Received 24 June 2013
Accepted 13 August 2013

ABSTRACT

(2*S*,3*R*)-Sitophilate, the male-produced aggregation pheromone of the granary weevil *Sitophilus granarius* (L.) was prepared stereoselectively using a novel chemoenzymatic approach in 50% overall yield. The synthetic design was based on an enantioselective fungal reduction of ethyl 2-methyl-3-oxopentanoate with a strain of *Aureobasidium pullulans* (CCM H1), followed by a Mitsunobu inversion at C3. The last step in the synthetic sequence was a lipase-mediated transesterification using the commercially available *Candida antarctica* B lipase (CaL B, Novozym 435) using microwave irradiation under solvent-free conditions.

1. Introduction

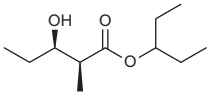
The granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) is a stored grain pest which causes serious economical losses throughout the world. Pest control has so far been carried out through the use of contact insecticides and fumigants, which have usually resulted in the development of insecticide-resistant strains.[1,2] This fact, together with environmental concerns, has contributed to promote the utilization of naturally occurring biological attractants such as pheromones for monitoring pest presence, thus allowing the implementation of integrated pest management programs. Phillips and Burkholder described in 1982 for the first time, evidence of a male-produced aggregation pheromone, attractive for both sexes in *S. granarius*.[3] The pheromone was later identified as (2*S*,3*R*)-1-ethylpropyl 3-hydroxy-2-methylpentanoate and called Sitophilate (Fig. 1).[4,5]

The first enantioselective synthesis of both enantiomers of Sitophilate was reported by Chong in 1989.[6] The synthetic strategy for the introduction of chirality involved a Sharpless asymmetric epoxidation of a *cis*-allylic alcohol, and further regioselective epoxide opening with Me_2CuLi, affording both (2*S*,3*R*)- and (2*R*,3*S*)-Sitophilate. Thereafter, several different synthetic pathways to either racemic or enantiomerically pure Sitophilate as well as its stereoisomers have been described,[7–17] most of which involved multistep reaction schemes and low yields. Recently, Smounou et al. described an efficient preparation of (2*S*,3*R*)-Sitophilate,[18] in high yield and enantiomeric purity. The synthetic strategy involved a stereoselective ketone reduction of methyl 2-methyl-3-oxopentanoate using commercial ketoreductases, followed by enzymatic hydrolysis of the methyl ester and further chemical esterification through a nucleophilic substitution reaction on 3-bromopentane.

Herein we report a concise and stereoselective chemoenzymatic synthesis of (2*S*,3*R*)-sitophilate exploiting the synthetic potential of enzymatic reactions in two key steps. The required stereochemistry at C2 and C3 was introduced through an enantioselective fungal reduction of ethyl 2-methyl-3-oxopentanoate, followed by a Mitsunobu inversion at C3. The last step in the synthetic sequence was a lipase-mediated transesterification with 3-pentanol, furnishing the final product in a single step.

2. Results and discussion

A retrosynthetic analysis of Sitophilate shows that it can be obtained through an enantioselective reduction of the corresponding 2-methyl-3-oxopentanoate ester, and further transesterification with 3-pentanol (Fig. 2).



(2*S*,3*R*)-Sitophilate

Figure 1. (2*S*,3*R*)-1-Ethylpropyl 3-hydroxy-2-methylpentanoate.

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0957-4166/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.tetasy.2013.08.004

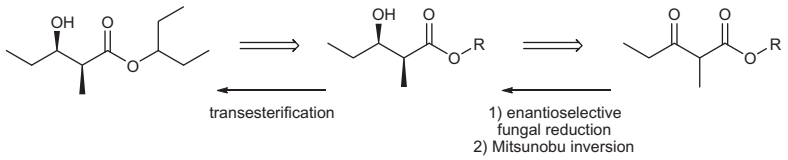
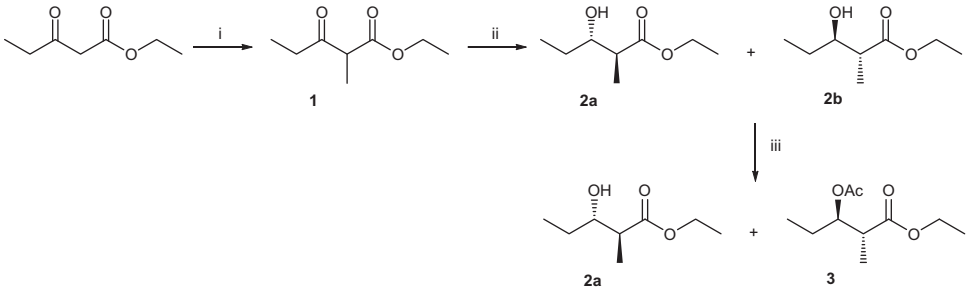


Figure 2. Synthetic design for the preparation of Sitophilate.

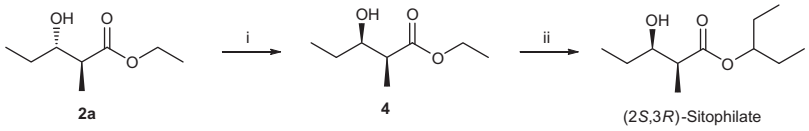
Racemic ethyl 2-methyl-3-oxopentanoate **1** was prepared from commercially available ethyl 3-oxopentanoate by alkylation with methyl iodide, according to the procedure described by Smounou et al. (Scheme 1).[18]

Stereoselective reduction of **1**, which contains both stereogenic and prochiral centers, was carried out through biotransformation using a strain of *Aureobasidium pullulans* (CCM H1), in agreement with our previous results based on an extensive screening of microorganisms as reducing agents for α-methyl-β-ketoesters.[19]

The fungal biotransformation was performed in an orbital shaker at 28 °C and 150 rpm (see Section 4). In order to determine the stereochemical profile of the transformation, aliquots were taken at 24, 48, 72, and 96 h. Despite the behavior described for 2-methyl-3-oxobutanoic esters, where high diastereo- and enantioselectivities were described,[19] we found for this reaction a high diastereoselection but moderate enantioselection. The best results were achieved at 72 h, giving 95% yield, with an *anti*:*syn* ratio of 99:1, and an enantiomeric excess of 64% for the (2*S*,3*S*)-isomer. For the assignment of the absolute configuration of the (2*R*,3*R*)- and (2*S*,3*S*)-isomers **2a** and **2b,** respectively, and the measurement of the *anti*/*syn* ratio, we compared them by chiral GC chromatography with the standards obtained by reduction of **1** according to the procedure described by Stewart et al.,[20] with the engineered yeast strains (Fasp++, Gre2p-, Ypr1p-; 24B(pSRG29)) ((*R*)-selective) and (Gre2p++, Fasp-; 2B(pSRG41)) ((*S*)-selective). In order to overcome the drawback of the moderate enantioselectivity of the reduction, we purified the (2*S*,3*S*)-enantiomer through a lipase-catalyzed kinetic resolution of the enantiomeric mixture, using lipase B from



Scheme 1. Chemoenzymatic synthesis of key intermediates to Sitophilate. Reagents and conditions: (i) K_2CO_3, CH_3I, dry acetone, reflux, 99% yield; (ii) *Aureobasidium pullulans* (CCM H1), 28 °C, 150 rpm, 95% yield, *anti*:*syn* ratio: 99:1; **2a**:**2b** ratio: 82:18; (iii) CaL B lipase, vinyl acetate, MW, 50 °C, 100% conversion of isomer **2b**, >99% ee for both **2a** and **3**.



Scheme 2. Chemoenzymatic synthesis of Sitophilate. Reagents and conditions: (i) (a) PNBA, PPh_3, DIAD, THF, rt, 72 h, (b) K_2CO_3, MeOH, rt, 65% for both steps; (ii) 3-pentanol, CaL B, MW, 50 °C, 1 h, 98% yield, >99% ee.

Table 1
Screening of commercial lipases for the transesterification of **3** with 3-pentanol

Entry	Strain	Conversion[a] (%)		
		24 h	48 h	72 h
1	*P. cepacia* (Lipase PS 'Amano')	21	40	48
2	*M. javanicus* (Lipase M 'Amano' 10)	8	16	41
3	*R. orizae* (Lipase F 'Amano' AP15)	—	—	7
4	*C. rugosa* (Lipase AY 'Amano' 30G)	—	16	25
5	*C. rugosa* (L-1754 lipase, SIGMA)	—	13	14
6	*P. camembertii* (Lipase G 'Amano' 50)	—	7	8
7	*P. roquefortii* (Lipase R 'Amano' G)	—	—	6
8	*A. niger* (Lipase A 'Amano' 12)	—	—	4
9	*C. antarctica* A (Chirazyme L5-Lyo)	40	59	75
10	*C. antarctica* B (Novozym 435)	87	95	98
11	*R. miehei* (Lipozyme RM-IM)	—	—	9
12	Pancreatic porcine lipase (L-3126 Lipase, SIGMA)	—	14	26

Reaction conditions: orbital shaking, 30 °C, 150 rpm, hexane as the solvent.
[a] Conversion is expressed as % of substrate transesterified, and was determined by GC analysis.

Table 2
Transesterification of ethyl 3-hydroxy-2-methylpentanoate with 3-pentanol

Entry	Lipase	Temperature (°C)	Solvent	Reaction time (h)	Conversion[a] (%)
1	*C. antarctica* A lipase (Chirazyme L5-Lyo)	30	Hexane	72	75
2			Solvent-free	24	77
3		40	Hexane	72	65
4			Solvent-free	24	70
5		50	Hexane	72	35
6			Solvent-free	24	40
7	*C. antarctica* B lipase (Novozym 435)	30	Hexane	72	98
8			Solvent-free	24	98
9		40	Hexane	72	75
10			Solvent-free	24	80
11		50	Hexane	72	40
12			Solvent-free	24	45

Reaction conditions: CaL A and CaL B as biocatalysts, orbital shaking, 150 rpm, 30, 40, and 50 °C, hexane as the solvent.
[a] Conversion is expressed as % of substrate transesterified, and was determined by GC analysis.

Candida antarctica (CaL B, Novozym 435) as the biocatalyst. Thus, vinyl acetate was selectively transesterified with compound **2b**, according to the Kazlauskas rule, and the remaining (2*S*,3*S*)-isomer **2a** was isolated quantitatively with >99% ee. Following previously described procedures, the reaction was performed at 50 °C under microwave irradiation in a solvent-free system.[21]

Upon preparation of **2a** in enantiopure form, it was subjected to a Mitsunobu inversion at C3 using *p*-nitrobenzoic acid (PNBA), followed by hydrolysis of the corresponding *p*-nitrobenzoate ester to give (2*S*,3*R*)-ethyl 3-hydroxy-2-methylpentanoate, **4**, in 70% yield and >99% ee (Scheme 2).

The last step in the synthetic sequence to Sitophilate was the enzymatic transesterification of **4** using 3-pentanol as the nucleophile. For this purpose, a screening of twelve commercial lipases was performed, using a diastereomeric mixture of ethyl 3-hydroxy-2-methylpentanoate as a model, which was prepared by the chemical reduction of **1** with $NaBH_4$.[19] The enzymatic reactions were carried out in an orbital shaker using hexane as the solvent, at 30 °C, 150 rpm, and taking aliquots at 24, 48, and 72 h. The results are shown in Table 1.

1-Ethylpropyl 3-hydroxy-2-methylpentanoate was obtained will all the enzymes screened, but good to excellent conversions were only found using lipases A and B from *C. antarctica* (CaL A and CaL B, entries 9 and 10, Table 1). With these results in hand, CaL A and CaL B were selected for further experiments in order to optimize the temperature and solvent conditions for our target product (Table 2).

As shown in Table 2, the solvent free conditions were always faster, showing better conversions in shorter times, at all temperatures assayed with both lipases. According to these results, the best results for the transesterification of (2*S*,3*R*)-ethyl 3-hydroxy-2-methylpentanoate with 3-pentanol as the nucleophile were obtained using CaL B lipase as the biocatalyst in the absence of a solvent, in an orbital shaker at 30 °C and 150 rpm. In this manner, (2*S*,3*R*)-Sitophilate was obtained with 98% yield in 24 h. According to the literature and our own experience, a synergism between enzyme catalysis and microwave irradiation can take place for some reactions.[21,22] On this basis, we conducted the reaction in a monomode microwave reactor under solvent-free conditions at 50 °C. After 1 h of irradiation, enantiopure (2*S*,3*R*)-Sitophilate was obtained in the same yield as with orbital shaking and conventional heating (98%). Hence, the natural pheromone of *S. granarius* was prepared through a synthetic route, which incorporates two enzymatic steps, providing for the overall process an environmentally benign profile. Moreover, the incorporation of an enzymatic kinetic resolution allowed the easy separation of an enantiomeric mixture of **2a** and **2b**. In addition, the whole process was scaled-up to 0.5 g of the final product in order to promote field evaluation.

3. Conclusion

A strain of *Aureobasidium pullulans* (CCM H1) and the commercially available lipase B from *Candida antactica* (Novozym 435) were used as biocatalysts in a novel and concise approach to give (2*S*,3*R*)-Sitophilate. The final product was obtained from simple and commercially available starting materials with an overall yield of 50%, and high stereomeric purity (99% de, >99% ee). Preliminary studies on scaling up the process were successfully carried out (up to 0.5 g), and further experiments on this topic are currently in progress, showing that an efficient method for the synthesis of this natural pheromone is provided.

4. Experimental

4.1. Chemicals and analysis

Solvents were purified and dried by conventional methods. Commercial reagents were purchased from Sigma–Aldrich Inc. The purity of the reactants, as well as the extent of the conversion of the reactions were controlled using analytical TLC on silica gel (Kieselgel HF 54 from Macherey-Nagel) and visualized with UV light (254 nm) and/or *p*-anisaldehyde in acidic ethanolic solution. Further analyses were performed by chiral gas chromatography (GC) in a Shimadzu 2010 equipment, with a FID detector (Flame Ionization Detector) and a Megadex DET-TBS (25 m × 0.25 mm × 0.25 μm) column. Temperature program: 60 °C/1 °C/min/70 °C (10 min)/2 °C/min/150 °C (5 min). T_{SPLIT}: 220 °C, T_{FID}: 250 °C. Non chiral gas chromatographies were conducted in a Shimadzu 2014 equipment with FID detector, and a Carbowax 20 MEGA (30 m × 0.25 mm × 0.25 μm) column. Temperature program: 60 °C (5 min)/8 °C/min/140 °C (0 min)/25 °C/min/240 °C (5 min). T_{SPLIT}: 220 °C, T_{FID}: 250 °C. The ee and absolute configuration of the reduction products were determined by chiral gas chromatography, and by comparison with standards. GC–MS (GC–mass spectrometry) analyses were performed using a QP-2010 Shimadzu GC–MS, equipped with a AT-5 MS column (Alltech) (30 m × 0.25 mm, 0.25 mm), operated with a constant carrier flow (He) of 1 mL/min. The temperature program was 70 °C (1 min)/7 °C/min, 250 °C. The injector temperature: 250 °C; the interphase temperature: 300 °C. Injection (1 mL) was in the split mode. EI-MS (electron ionization-MS) were obtained in the scan mode from *m*/*z* 40 to 350. Column chromatography was performed using flash silica gel (Kieselgel 60, EM reagent, 230–240 mesh) from Macherey-Nagel. NMR spectra (^1H and ^{13}C) were carried out in a Bruker Avance DPX 400 MHz equipment. All experiments were carried out at 30 °C, $CDCl_3$ was used as solvent, and TMS as internal stan-

dard. Optical rotations were measured on a Zuzi 412 polarimeter using a 0.5 m cell. $[\alpha]_D$ values are given in units of deg cm^2 g^{-1} and concentration values are expressed in g/100 mL. Biotransformations were conducted in orbital shakers from Thermoforma (model 420) and Sanyo (model IOX400.XX2.C). The microwave heating was performed in a laboratory monomode microwave reactor (CEM Corporation, Discover model) using 10 mL Pyrex tubes equipped with septum seal and magnetic stirring. The strain of *A. pullulans* (CCM H1) belongs to the collection of the Microbiology Laboratory of the Biosciences Department (Facultad de Química, UdelaR) and is freely available upon request.

4.2. Racemic ethyl 2-methyl-3-oxopentanoate 1

Ethyl 2-methyl-3-oxopentanoate was prepared from commercially available ethyl 3-oxopentanoate by alkylation with methyl iodide, according to the procedure described by Smounou et al.[18] Ethyl 3-oxopentanoate (0.22 g, 2.0 mmol) was dissolved in anhydrous acetone (2 mL) under a nitrogen atmosphere. Dried potassium carbonate (0.2 g, 1 mmol) was added with magnetic stirring. The solution was maintained at room temperature for 10 min, then methyl iodide (0.12 mL, 2 mmol) was added with a syringe. The reaction was refluxed for 12 h. After the reaction was complete, diethyl ether was added (3 mL), and the mixture was filtered. The solvent was distilled under vacuum, and the residue was purified by flash chromatography (silica gel, Hex/AcOEt, v/v: 95:5) giving compound **1** (0.31 g, 99%) as a pale yellow oil. Chiral GC: t_R = 18.27 min, 19.44 min. ^{1}H NMR: δ (ppm) = 1.04 (t, J = 7.2 Hz, 3H, CH_3), 1.23 (t, J = 7.1 Hz, 3H, CH_3), 1.30 (d, J = 7.1 Hz, 3H, CH_3), 2.49 (dq, J_1 = 7.2 Hz, J_2 = 18.2 Hz, 1H, CH_2), 2.59 (dq, J_1 = 7.2 Hz, J_2 = 18.2 Hz, 1H, CH_2), 3.53 (q, J = 7.1 Hz, 1H, CH), 4.15 (q, J = 7.1 Hz, 2H, CH_2). ^{13}C NMR: δ (ppm) = 7.6, 12.8, 14.0, 34.6, 52.5, 61.2, 170.7, 206.5.

4.3. Ethyl 3-hydroxy-2-methylpentanoate 2a,2b

The culture medium was prepared according to the literature,[23] containing (g/l): sucrose (50), $(NH_4)_2SO_4$ (5), $CaCl_2{\cdot}2H_2O$ (0.25), and $MgSO_4{\cdot}7H_2O$ (0.25). The culture medium (50 mL) was inoculated in a 125 mL flask, with 5 mL of spores suspension (approx. 10^7 cfu/mL) obtained from fresh cultures of *Aureobasidium pullulans* (CCM H1) in PDA plates at 25 °C. Cultures were incubated for 3 days at 28 °C with orbital shaking (150 rpm), then 50 mg of ethyl 2-methyl-3-oxopentanoate **1** was added and incubated for 96 h at 28 °C with shaking. Reduction products of ethyl 2-methyl-3-oxopentanoate were extracted from the culture medium with ethyl acetate (3 × 5 mL), and the organic layer was dried over anhydrous $MgSO_4$. The desiccant agent was filtered, and ethyl acetate was distilled under vacuum on a rotary evaporator at 30 °C. The residue was taken up in CH_2Cl_2, and aliquots containing 1 mg/mL of reduction products were analyzed by GC using the conditions described in Section 4.1. Yield: 0.048 g, 95%. Compound **2** was obtained as a pale yellow oil, with an *anti*:*syn* ratio: 99:1, (2*S*,3*S*)/(2*R*,3*R*) ratio: 82/18. Chiral GC: t_R(**2a**): 23.67 min; t_R(**2b**): 23.33 min.

4.4. Purification of (2*S*,3*S*)-ethyl 3-hydroxy-2-methylpentanoate 2a by enzymatic derivatization of (2*R*,3*R*)-ethyl 3-hydroxy-2-methylpentanoate

The enantiomeric mixture of **2a** and **2b** (0.62 g, 3.9 mmol) in a ratio **2a**/**2b** 82:18, lipase B from *C. antarctica* (Novozym 435, 0.2 g), vinyl acetate (1 g, 12 mmol), and hexane (2 mL) as the solvent were heated at 50 °C in a microwave reactor for 2 h. The enzyme was then filtered, the solvent was distilled under reduced pressure, and the crude was purified by flash chromatography (silica gel, Hex/AcOEt, v/v: 9:1) giving **2a** (0.05 g, 3.1 mmol, >99% ee) as a pale oil, and **3** (0.14 g, 0.69 mmol, >99% ee). Chiral GC: t_R(**2a**): 23.67 min; t_R(**3**): 26.04 min. **2a**: ^{1}H NMR: δ (ppm) = 0.99 (t, J = 7.4 Hz, 3H, CH_3), 1.22 (d, J = 7.2 Hz, 3H, CH_3), 1.29 (t, J = 7.2 Hz, 3H, CH_3), 1.47 (m, 1H, CH_2), 1.59 (ddq, J_1 = 13.9 Hz, J_2 = 7.5 Hz, J_3 = 4.0 Hz, 1H, CH_2), 2.54 (dq, J_1 = 7.2 Hz, J_2 = 6.4 Hz, 1H, CH), 3.60 (ddd, J_1 = 8.3 Hz, J_2 = 6.3 Hz, J_3 = 4.0 Hz, 1H, CH), 4.18 (q, J = 7.2 Hz, 2H, CH_2). ^{13}C NMR: δ (ppm) = 9.8, 13.2, 14.2, 27.6, 44.8, 60.6, 74.7, 176.2.

4.5. (2*S*,3*R*)-Ethyl 3-hydroxy-2-methylpentanoate 4

(2*S*,3*S*)-Ethyl 3-hydroxy-2-methylpentanoate **2a** (0.18 g, 1.1 mmol), triphenylphosphine (0.39 g, 1.5 mmol), and *p*-nitrobenzoic acid (0.25 g, 1.5 mmol) were stirred in dried THF in a round bottom flask under a nitrogen atmosphere. Next, diisopropylazodicarboxilate (DIAD, 0.3 g, 1.5 mmol) was added dropwise and the reaction was stirred at room temperature for 72 h. The solvent was distilled off under reduced pressure, and the crude was purified by flash chromatography (silica gel, Hex/AcOEt, v/v: 95:5). The obtained *p*-nitrobenzoic ester (0.146 mg, 0.47 mmol) was dissolved in the minimum absolute EtOH (3 mL) and catalytic amounts of K_2CO_3 were then added. The reaction mixture was stirred for 96 h, then filtered, and the solvent was distilled under reduced pressure. The crude was purified by flash chromatography (silica gel, Hex/AcOEt, v/v: 95:5) giving **4** (0.126 g, 0.79 mmol, 65% for both steps) as a pale oil. $[\alpha]_D^{25} = +15.0$ (c 2.8, MeOH). Chiral GC: t_R: 22.48 min. ^{1}H NMR: δ (ppm) = 0.99 (t, J = 7.4 Hz, 3H, CH_3), 1.22 (d, J = 7.2 Hz, 3H, CH_3), 1.29 (t, J = 7.2 Hz, 3H, CH_3), 1.50 (m, 1H, CH_2), 1.58 (m, 1H, CH_2), 2.56 (m, 1H, CH), 3.83 (m, 1H, CH), 4.18 (q, J = 7.3 Hz, 2H, CH_2). ^{13}C NMR: δ (ppm) = 9.8, 14.2, 14.4, 27.6, 44.8, 60.6, 74.7, 176.2.

4.6. Screening of commercial lipases as catalysts in the the transesterification of ethyl 3-hydroxy-2-methylpentanoate with 3-pentanol

Twelve commercial lipases from *Pseudomonas cepacia*, *Mucor javanicus*, *Rhizopus oryzae*, *Candida rugosa*, *Penicillium camembertii*, *Penicillium roquefortii*, *Aspergillus niger*, *Rhizomucor miehei*, *C. antarctica* A and B and pancreatic porcine lipase (PPL) were screened for the final step in the synthetic sequence to Sitophilate. A diastereomeric mixture of ethyl 3-hydroxy-2-methylpentanoate (0.05 g, 0.35 mmol) obtained from the chemical reduction of racemic ethyl 2-methyl-3-oxopentanoate **1** with $NaBH_4$[19] was used as the substrate. The reactions were carried out in an orbital shaker at 30 °C and 150 rpm with hexane (2 mL) as the solvent, (0.046 g, 0.53 mmol) of 3-pentanol and 20 mg of the corresponding lipase. Aliquots were taken at 24, 48, and 72 h and analyzed by GC in a carbowax column. Secondary screening was conducted using *C. antarctica* A and B lipases varying the temperature to 40 and 50 °C, in hexane and in solvent-free conditions, maintaining the other experimental conditions unchanged. The optimization of the protocol for the transesterification of ethyl 3-hydroxy-2-methylpentanoate with 3-pentanol was conducted under microwave irradiation at 50 °C and in the absence of solvent.

4.7. (2*S*,3*R*)-Sitophilate

(2*S*,3*R*)-Ethyl 3-hydroxy-2-methylpentanoate **3** (0.2 g, 1.25 mmol), 3-pentanol (0.132 g, 1.5 mmol), and lipase B from *C. antarctica* (Novozym 435, 0.05 g) were heated at 50 °C in a microwave reactor for 1 h. The enzyme was filtered and the solvent was distilled under reduced pressure. The crude was purified by flash chromatography (silica gel, Hex/AcOEt, v/v: 7:3) giving Sitophilate (0.247 g, 98%, >99% ee) as a pale yellow oil. $[\alpha]_D^{22} = -5.4$ (c 1.0, AcOEt). GC (Carbowax 20 column): t_R: 17.71 min. ^{1}H NMR: δ

(ppm) = 0.90 (t, J = 7.4 Hz, 3H, CH_3), 0.91 (t, J = 7.4 Hz, 3H, CH_3), 1.0 (t, J = 7.4 Hz, 3H, CH_3), 1.21 (d, J = 7.3 Hz, 3H, CH_3), 1.54 (m, 2H, CH_2), 1.59 (m, 4H, CH_2), 2.56 (dq, J_1 = 7.3 Hz, J_2 = 3.5 Hz, 1H, CH), 3.83 (m, 1H, CH), 4.81 (m, 1H, CH). ^{13}C NMR: δ (ppm) = 9.4, 9.6, 10.4, 10.7, 26.4, 26.5, 26.6, 44.0, 73.2, 76.7, 176.3. MS (IE, 70 eV), m/z (%): 203 (<1, M^++1), 115 (51, M^+-OCH(CH_2CH_3)$_2$), 103 (27), 85 (12, M^+-OCH(CH_2CH_3)$_2$,CH_3,CH_3), 74 (100, $C_3H_6O_2^+$), 57 (27, $C_3H_5O^+$), 43 (30, $C_2H_2O^+$). (All experimental data were in agreement with those reported in the literature).[18]

Acknowledgments

The authors wish to thank the Microbiology Laboratory (Facultad de Química, Universidad de la República, Uruguay) for the generous gift of the strain of *A. pullulans*, as well as Professor Peter Wipf (Pittsburgh University) for his kind donation of a laboratory microwave reactor, and to Dr. Graciela Mahler for helpful discussions. Thanks are also due to Novozymes Inc. and AMANO Enzyme Inc. for the generous gift of lipases. Support for this work from DINACYT (PDT 77/22, Uruguay), OPCW (Organization for the Prohibition of Chemical Weapons), ANII (Agencia Nacional de Investigación e Innovación), and PEDECIBA (Programa de Desarrollo de las Ciencias Básicas) is gratefully acknowledged.

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