Enzyme-Mediated Preparation of the Single Enantiomers of the Olfactory Active Components of the Woody Odorant *Timberol*®

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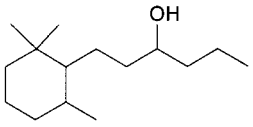
Enantiomerically pure (3*S*)-**3a** and -**3b**, the olfactory active forms of 1-(2,2,6-trimethylcyclohexyl)hexan-3-ol, components of the commercial woody odorant *Timberol*®, are obtained by lipase-PS-mediated enantioselective acetylation of the allylic alcohols **6** and **7** and of the saturated alcohol **3**. These materials, as mixtures of diastereoisomers, provided (3*R*)-configured transformation products. However, whereas in the conversion of **6** and **7** there is no diastereoselection, **3** provided the acetate of (1′*S*,3*R*,6′*R*)-**3c** much more rapidly than that of the diastereoisomer (1′*R*,3*R*,6′*S*)-**3d** (*Scheme 3*). Inversion of the configuration at C(3) of the side chain of the olfactory inactive (3*R*)-materials obtained as acetates in the enzymic treatment of **6**, **7**, and **3** also provided, eventually, the desired olfactory active (3*S*)-products.

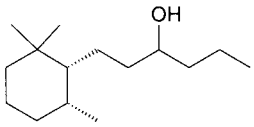
Introduction. – The structure-odour relationship is the subject of active current research interests [1][2][3a]. A relevant example of the evolution of the knowledge of the structural factors governing the still largely obscure phenomenon of olfaction through chemical synthesis is 1-(2,2,6-trimethylcyclohexyl)hexan-3-ol (**1**). The material was first put on the market nearly 20 years ago as *Timberol*® by *Dragoco* [4], a fragrance synthetic with fixative properties. The product, showing a powdery-woody odour with animal, steroid-type undertones, was soon introduced as a key component in many formulations of commercial success [2]. Subsequent studies indicated that the commercial product was mainly composed of the two racemic ring *cis*/*trans* diastereoisomers **2** (64%) and **3** (13%), respectively, and, most importantly, that the woody animal note was mainly due to the *trans* diastereoisomer-**3** [5], with the substituents at C(1′) and C(6′) of the cyclohexane moiety both in equatorial positions. The observed **2** (*cis*)/**3** (*trans*) ratio of *Timberol*® originates from the industrial synthesis, *i.e.*, the catalytic hydrogenation of the ionone homologue **4** (in mixture with the Δ^5-isomer).

The odour-active 1′,6′-equatorially disubstituted material **3** was subsequently prepared as a 1:1 mixture of the two racemic diastereoisomers **3a**,**c** and **3b**,**d**, and commercialized by *Firmenich* with the brand name of *Norlimbanol*®. This synthesis was based on the reduction of the dihydroionone analogue **5** possessing the desired 1′,6′-*trans* substitution due to the synthetic sequence starting from the *trans*-dihydrocyclocitral (=*trans*-2,2,6-trimethylcyclohexanecarboxaldehyde).

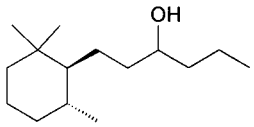
The single enantiomers **3a**–**d**, which are the components of *Norlimbanol*®, were later prepared, starting from the C_{10} and C_5 chiral pool components [6] (*R*)- and (*S*)-dihydrocyclocitral and L- and D-norleucine. Intermediates in the convergent synthesis of **3a**–**d** were the enantiomers of the alcohol prepared by reduction of *trans*-dihydrocyclocitral, and propyloxirane. The coupling of the two fragments was achieved



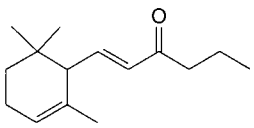
1



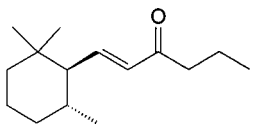
2 *cis*
(four stereoisomers)



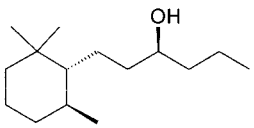
3 *trans*
(four stereoisomers)



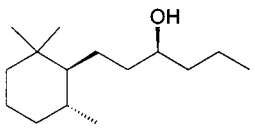
4 *rac*



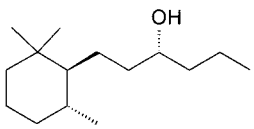
5 *rac*



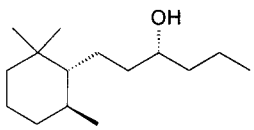
3a (1'*R*,3*S*,6'*S*)



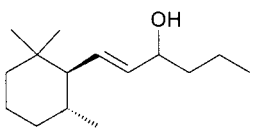
3b (1'*S*,3*S*,6'*R*)



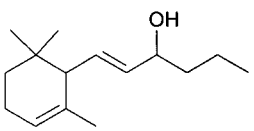
3c (1'*S*,3*R*,6'*R*)



3d (1'*R*,3*R*,6'*S*)



6 *rac*
(four stereoisomers)



7 *rac*
(four stereoisomers)

by reacting the phenylsulfone of the alcohol with the propyloxirane, the final step being the reductive desulfurization of the C_{15} adduct [7]. The olfactory evaluation of products **3a**–**d**, rather surprisingly, indicated that the two (3*S*)-diastereoisomers **3a** and **3b** are equally odour-active [5][3b], whereas the (3*R*)-materials **3c** and **3d** are devoid of odour.

These synthetic studies indicate that the olfactory response of **1** depends upon the equatorial position of the ring substituents and upon the absolute configuration at C(3), irrespectively of the absolute configuration at the ring C-atom bearing the side chain. This particular olfactory activity has been tentatively attributed to the role of the flexible side chain in the interaction with the receptors [3b].

In simple numerical terms, these results also indicate that the odour-active species present in *Timberol*® and *Norlimbanol*® account to 6.5 and 50% of the whole mixtures. This consideration makes of potential interest the preparation of the single olfactory active (3*S*)-components **3a** and **3b** on a way which should be more direct than the one reported, based on the use of rather expensive enantiomerically pure starting materials. Of higher practical potential seems to be the separation of the (3*S*)-stereoisomers **3a** and **3b** from those possessing (3*R*)-configuration, followed by the conversion of the latter into the former olfactory active diastereoisomers by inverting the configuration at the OH-bearing C-atom. This seemed accessible by enzymic methods based upon our recent observation [8][9] that the enantiomers of C_{13} α-ionol and of α- and β-ionol epoxide can be easily separated through lipase-mediated acetylation of the racemic materials.

Accordingly, as a possible entry to **3a** and **3b**, we explored the lipase-mediated acetylation of the allylic alcohols **6** and **7**, intermediates in the synthesis of racemic **3**, and of the saturated alcohol **3**. We report herein the results of these studies.

Results and Discussion. – The syntheses of the required substrates **3**, **6**, and **7** were achieved by the reported procedures [5][10]. Alcohol **6** was obtained as a 1:1 mixture of two racemic diastereoisomers by $NaBH_4$ reduction of **5** [5], while catalytic hydrogenation of the latter, followed by $NaBH_4$ reduction, provided **3**. The α-ionol analogue **7** was instead prepared as a 1:1 mixture of two diastereoisomers by $NaBH_4$ reduction of **4**, prepared, in turn, by condensing α-cyclocitral with pentan-2-one [10].

The diastereoisomer as well as the enantiomer composition of all the alcohols involved in the present study was determined by GC analysis of the corresponding acetates on a permethylated β-cyclodextrin column. Four peaks each were resolved for the acetates of the racemic diastereoisomers **3** and for the racemic diastereoisomers **6**. Three peaks (1:1:2) were obtained when the racemic diastereoisomers **7** were analysed.

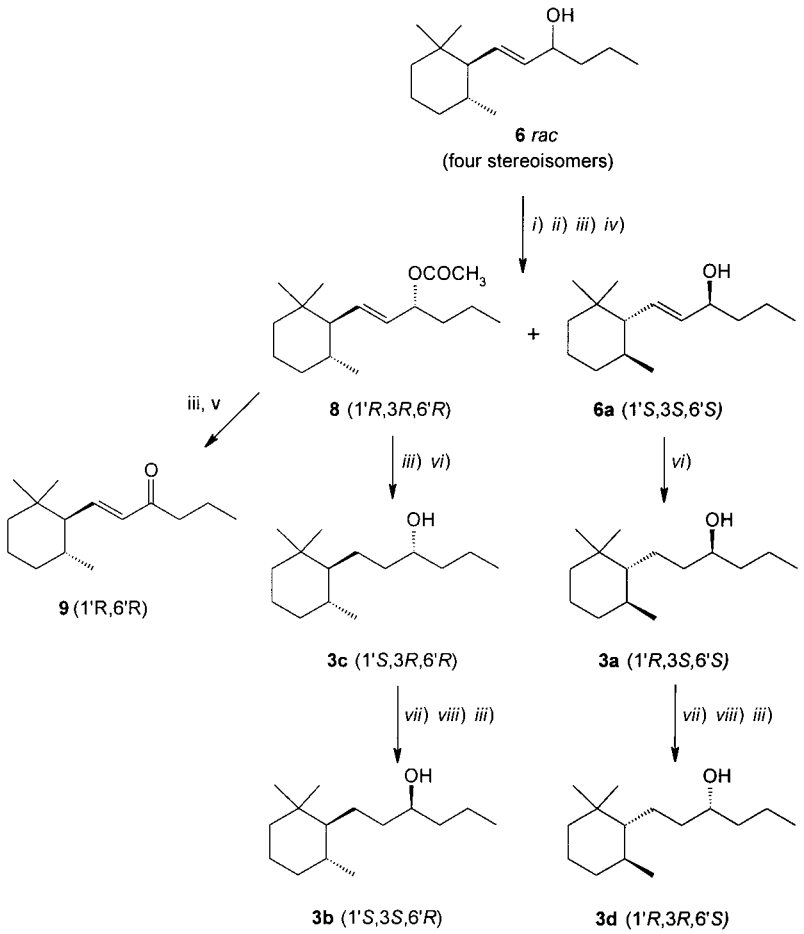
Exploratory enzymic acetylation experiments of substrates **3**, **6**, and **7** using vinyl acetate as an acetate donor in *tert*-butyl methyl ether (^{t}BuOMe) solution, in the presence of porcine pancreatic lipase (PPL) (*Sigma*), *Candida rugosa* lipase (CCL) (*Sigma*), and lipase PS (*Pseudomonas cepacia*) (*Amano*), respectively, indicated that only the latter accepted these substrates. However, differences in the mode of transformation of unsaturated **6** and **7** with respect to that of the fully hydrogenated derivative **3** occurred.

Lipase PS mediated the rapid acetylation of allylic alcohol **6**. GC Analysis of the acetylated material and of the unconverted alcohol sample, separated by column chromatography (CC; SiO_2), indicated that the former consisted of the species providing the 2nd and 4th peaks in the GC trace of **6** (as acetates), while the unconverted material consisted of the products eluted as 1st and 3rd peak. These products showed a negligible optical rotation in $CHCl_3$. The transformed and the unconverted materials were tentatively considered to be 1:1 mixtures of the enantiomerically pure diastereoisomeric (3*R*)-acetates and (3*S*)-alcohols, respectively, on the basis of the following evidences. Catalytic hydrogenation of the allylic alcohol **6** recovered from the enzymic acetylation (1st and 3rd GC peaks) indeed provided a strongly odoriferous product, identical by ^{1}H- and ^{13}C-NMR studies and GC/MS comparison (non-chiral column) to product **3**, whereas the enzymically acetylated material (2nd and 4th GC peaks) gave rise, by hydrolysis and hydrogenation, to odourless **3**. Thus, the lipase-PS-mediated acetylation of **6** proceeded with high enantioselectivity, but without diastereoselection.

The two racemic diastereoisomers of alcohol **6** were accordingly separated by fractional crystallization of their 4-nitrobenzoates (*Scheme 1*). The GC analyses of the acetate derivatives of **6** recovered from the less soluble 4-nitrobenzoate (three crystallizations from MeOH) and from the mother liquor are reported in the *Figure*. The racemic alcohol diastereoisomer recovered from the crystalline 4-nitrobenzoate (*Fig.*,*b*, 3rd and 4th GC peaks) provided, upon acetylation in the presence of lipase PS, the (1′*R*,3*R*,6′*R*)-acetate **8** (*Fig.*,*c*) and (1′*S*,3*S*,6′*S*)-alcohol **6a** (*Fig.*,*d*) which were separated by CC (SiO_2). The configurational assignment shown in *Scheme 1* was based on the conversion of **8** into the known [11] unsaturated ketone **9** by basic hydrolysis and MnO_2 oxidation, and of **6a** into (1′*R*,3*S*,6′*S*)-alcohol **3a** (*cf.* [7]), showing an extremely pleasant powdery woody odour, by catalytic hydrogenation (H_2, PtO_2, AcOH). The allylic alcohol obtained upon basic hydrolysis of **8** was similarly hydrogenated to (1′*S*,3*R*,6′*R*)-alcohol **3c** (*cf.* [7]). Subsequently, products **3a** and **3c** were converted separately to the corresponding 4-toluenesulfonates, providing, upon displacement by acetate in refluxing dimethylformamide (DMF) and basic hydrolysis, the (3*R*)- and (3*S*)-configured diastereoisomers **3d** and **3b**, respectively, showing optical rotations in agreement with those reported [7]. This sequence thus completed the enzyme-mediated preparation of the two enantiomers of the olfactory active diastereoisomers present in *Timberol*® (**1**) [4] from the racemic unsaturated ketone **5**, intermediate in the synthesis of *Norlimbanol*® (**3**) [5].

Allylic alcohol **7**, obtained as a 1:1 mixture of two racemic diastereoisomers, was the next substrate to be submitted to lipase PS acetylation. The use of suitable enantiomerically pure stereoisomers of derivative **7** as precursors of valuable **3a** and **3b** appeared at first of limited value, since catalytic hydrogenation of the cyclohexenyl moiety of **7** was known [5] to provide the undesired *cis*-alcohol **2** as the major diastereoisomer. We investigated the reduction of racemic **7** under different reaction conditions (Pd/C in AcOH, Pd/C in EtOH, Pd/C in cyclohexane, PtO_2 in AcOH), evaluating the *cis*/*trans* ratio by means of GC (t_R 13.31 (**3** (*trans*)) and 13.48 min (**2** (*cis*)) on non-chiral column, see *Exper. Part*) and ^{1}H-NMR analyses. The study of the ^{1}H-NMR spectra was rather interesting, since H−C(1′) of the cyclohexane ring resonated at 0.51 ppm (*ddd*) in **3** (*trans*), while it was shifted downfield and overlapped

Scheme 1



i) 4-Nitrobenzoyl chloride, CH_2Cl_2, pyridine. *ii*) Three crystallizations from MeOH. *iii*) NaOMe in MeOH. *iv*) Vinyl acetate, ^{t}BuOMe, lipase PS, column chromatography. *v*) Activated MnO_2 in CH_2Cl_2. *vi*) H_2, PtO_2, AcOH. *vii*) TsCl, pyridine. *viii*) NaOAc, DMF, reflux.

with many other signals in **2** (*cis*). The *cis*/*trans* composition was thus evaluated by the ratio of the integrals of the signals at 3.56 ppm (C*H*OH of both **2** and **3**) and at 0.51 ppm.

In our hands, catalytic hydrogenation of racemic **7** in the presence of 10% Pd/C in AcOH provided **2**/**3** in *ca.* 6:4 ratio, besides variable amounts of the C_{15} hydrocarbon homologue of perhydroionane, formed by hydrogenolysis of the O–C bond in the allylic position. This result, combined with the observation that CC (SiO_2) of the 6:4 hydrogenation mixture allowed us to obtain the *trans* diastereoisomer **3** in fair yield as the first eluted product, encouraged us in this study. The outcome of this investigation is illustrated in *Scheme 2*. Thus, enzymic acetylation of alcohol **7** (three GC peaks for the corresponding acetates in a 1:1:2 ratio) provided an acetate derivative (2nd and 3rd GC peaks) which was assumed to be a 1:1 mixture of the two enantiomerically pure

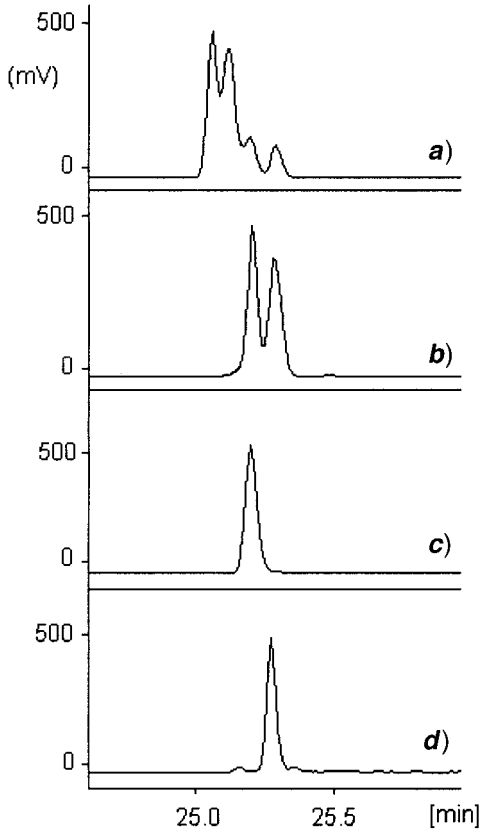
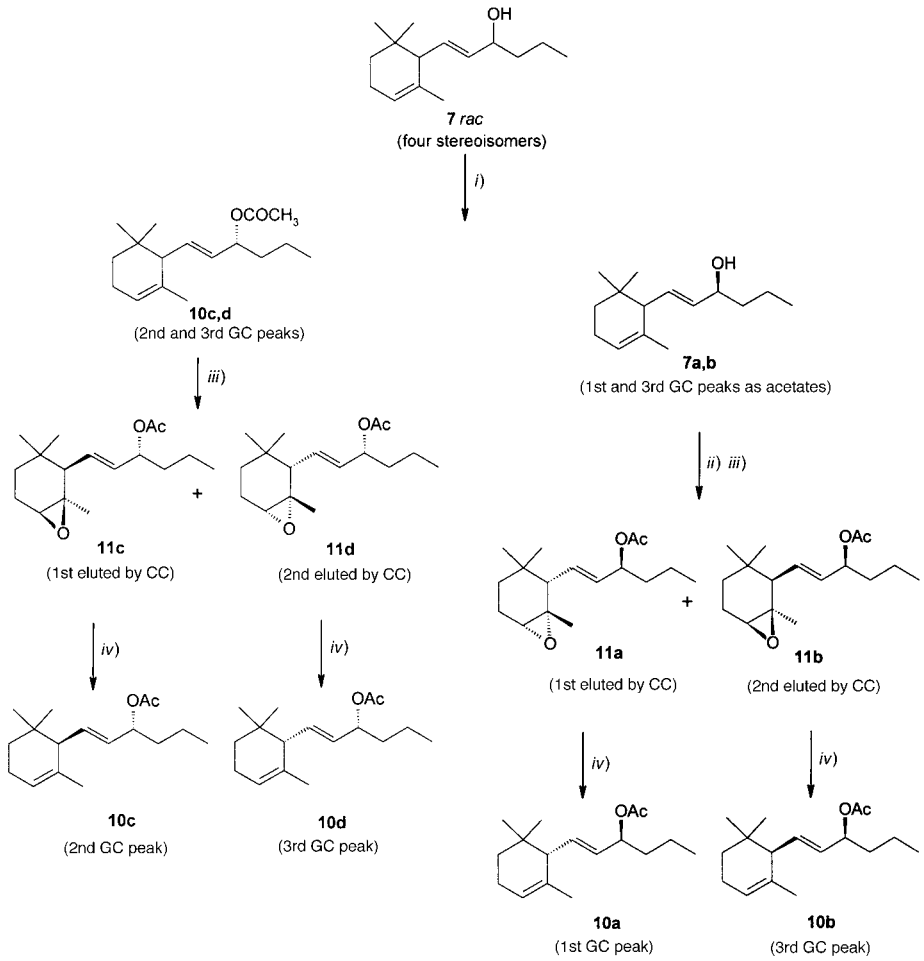


Figure. *Chiral GC analyses of* a) *the acetate derivative of alcohol* **6** *recovered from the mother liquors of the crystallization of the 4-nitrobenzoates*; b) *acetate derivative of alcohol* **6** *recovered from the crystalline 4-nitrobenzoate*; c) *(1′*R,*3*R,*6′*R*)-acetate* **8**; d) *acetate derivative of (1′*S,*3*S,*6′*S*)-alcohol* **6a**

(3*R*)-diastereoisomers **10c** and **10d**, since we had observed that lipase PS preferentially reacted with (*R*)-allylic alcohols [8][9]. The unconverted alcohol was considered to be a 1:1 mixture of the two (3*S*)-diastereoisomers **7a** and **7b** (1st and 3rd GC peaks as acetates). This assumption was confirmed at the end of the synthetic sequence. We did not investigate the absolute configuration at the stereogenic centre of the cyclohexane moiety, since for our purposes, only the configuration at C(3) was of interest for the olfactory response of saturated product **3**. Thus, the absolute configuration of the C-atom bearing the side chain shown in *Scheme 2* is tentatively attributed.

Substrates **10c**,**d** and **7a**,**b** were then converted to the corresponding epoxy derivatives by treatment with 3-chloroperbenzoic acid in CH_2Cl_2 (*Scheme 2*); we assumed the formation of the *cis*-epoxide derivative as the main product in both cases, as it was known to happen for the epoxidation of α-ionol under the same reaction conditions [9]. Column chromatography of the two mixtures of diastereoisomeric epoxy acetates allowed us to isolate the four stereoisomers **11a**–**d** as pure compounds (see *Exper. Part*). Substrates **11a**–**d** were deoxygenated separately to afford derivatives

Scheme 2

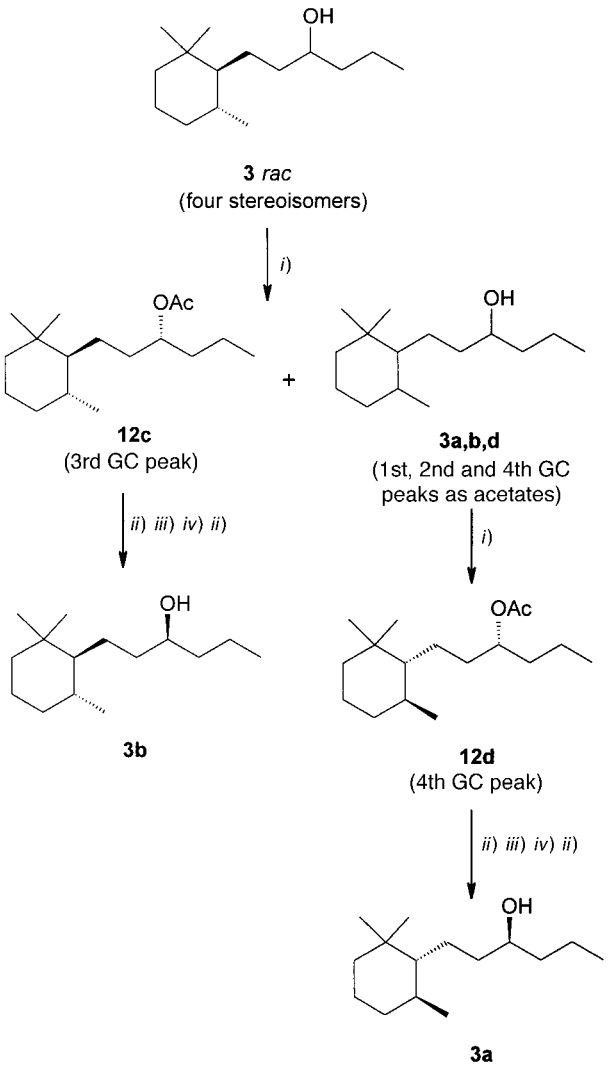


i) Vinyl acetate, ^{t}BuOMe, lipase PS, column chromatography. *ii*) Ac_2O, pyridine. *iii*) 3-Chloroperbenzoic acid, CH_2Cl_2, column chromatography. *iv*) Zn, NaI, NaOAc, AcOH.

10a–**d** by treatment with Zn/NaI/AcONa in AcOH [12] (**10a**: 1st GC peak, ee >99%, de 71%; **10b**: 3rd GC peak, ee >99%, de 90%; **10c** 2nd GC peak, ee >99%, de 91%; **10d**: 3rd GC peak, ee >99%, de 99%). Thus, this synthetic sequence yielded another set of optically active precursors of the four stereoisomers **3a**–**d**, allowing to verify the great potential of this combination of enantioselective enzymic reactions with chemical separation of diastereoisomers. However, for the aim of this work, only the 1 : 1 mixture of the unconverted (3*S*)-alcohols **7a**,**b** was hydrogenated in AcOH in the presence of 10% Pd/C to provide the two olfactory active stereoisomers of **3**, after column chromatography.

Saturated alcohol **3** showed a completely different behaviour (*Scheme 3*) when it was treated with lipase PS in the presence of vinyl acetate in ^{t}BuOMe solution. The

Scheme 3



i) Vinyl acetate, ^{t}BuOMe, lipase PS, column chromatography. *ii*) NaOMe, MeOH. *iii*) TsCl, pyridine. *iv*) NaOAc, DMF, reflux.

acetate derivative obtained after 24 h was composed mainly of stereoisomer **12c** (3rd GC peak, ee $>99\%$, de $=81\%$) with only 9% of the other (3*R*)-diastereoisomer derived from **3d** (4th GC peak). Thus, this lipase-mediated acetylation resulted to be enantiospecific and highly diastereoselective. The alcohol mixture recovered from this first biocatalyzed reaction, which was almost completely deprived of the stereoisomer whose acetate **12c** gave the 3rd GC peak, was submitted again to enzymic acetylation. After 7 days, acetate derivative **12d** (4th GC peak, ee $>99\%$, de 95%) was isolated from the reaction mixture by CC. The two diastereoisomers **12c** and **12d** showed very

similar ^{13}C-NMR spectra; however, the small differences (maximum 0.17 ppm) observed for many C-atoms could be used to determine the diastereoisomer enrichment of each stereoisomer in a way alternative to that offered by GC. In separate experiments, compounds **12c** and **12d** were hydrolyzed, converted to the corresponding tosylate derivatives, and submitted to acetate displacement, to yield, after saponification, the enantiomerically enriched (3*S*)-substrates **3b** and **3a**.

Conclusion. – Three different synthetic approaches to enantiomerically enriched derivatives **3a** and **3b**, the olfactory active components of the woody odorant *Timberol*®, were shown in this work.

The enantiospecific enzyme-mediated acetylations of allylic alcohols **6** and **7** were combined with physical methods for the separation of diastereoisomers, *i.e.* fractional crystallization of the 4-nitrobenzoates for substrate **6** and column chromatography of epoxy alcohol derivatives for substrate **7**. In both cases, enantiomerically pure precursors of all the four stereoisomers of substrate **3** (*i.e.*, **3a**–**d**) were obtained. Unexpectedly, when substrate **3** itself (four stereoisomers) was submitted to lipase acetylation, not only enantiospecificity, but also high diastereoselectivity was found. As for our experience, the steric course of lipase-PS-mediated acetylation of structurally similar derivatives, such as α-ionol [8], epoxy α- and β-ionol [9], allylic alcohols **6** and **7**, and saturated alcohol **3**, is almost unpredictable. The reaction is usually highly enantioselective (with a great preference for the acetylation of (*R*)-alcohols), but not diastereoselective at all, except in the case of derivative **3**. A slow biocatalysed reaction of **3** afforded acetate **12c** showing a de of 81% (**12c**/**12d** 91:9 by GC). Moreover, for the sake of completeness, we submitted a homologue of racemic **7**, bearing one more Me group at C(5), to lipase-mediated acetylation. The growth of the steric hindrance around the OH group completely inhibited biocatalyzed acetylation.

Experimental Part

General. The following enzymes were employed in this work: *Candida cylindracea* lipase (*Sigma*, type VII, 900 U/mg), porcine-pancreas lipase (*Sigma*, type II), and lipase PS *Pseudomonas cepacia* (*Amano Pharmaceuticals Co.*, Japan). TLC: *Merck* silica gel *60 F_{254}* plates. Column chromatography (CC): silica gel. GC: *DANI-HT-86.10* gas chromatograph; enantiomer and diastereoisomer excesses were determined on a *Chirasil DEX CB* (*Chrompack*) column (25 m × 0.25 mm) with the following temp. program: 70° (3 min) → 3.5°/min → 140° → 8°/min → 180° (1 min); the ratio **2** (*cis*)/**3** (*trans*) was evaluated on a *Megawax* column (25 m × 0.25 mm) with the following temp. program: 50° (5 min) → 8°/min → 120° (2 min) → 2°/min → 170° (1 min) → 20°/min → 200°; t_R in min. $[\alpha]_D$: *Jasco-DIP-181* digital polarimeter. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-AC-250* spectrometer (250 MHz (^{1}H)); $CDCl_3$ solns. at r.t., unless otherwise stated; chemical shifts δ in ppm rel. to internal $SiMe_4$, *J* values in Hz. Microanalyses: *Carlo-Erba 1106* analyser.

(±)-1-(2,2,6-Trimethylcyclohexyl)hex-1-en-3-ol (**6**; 4 stereoisomers). $NaBH_4$ (5.14 g, 0.135 mol) reduction of racemic **5** (20 g, 0.09 mol; prepared according to [5]) in CH_2Cl_2/MeOH 1:1 (200 ml) afforded, after purification by CC (hexane/AcOEt 7:3), **6** (19.1 g, 95%) as a 1:1 mixture of two racemic diastereoisomers. Chiral GC: t_R 25.03, 25.10, 25.18, 25.27 [1]. ^{1}H-NMR: 5.37 (*m*, H–C(1), H–C(2)); 4.10 (*q*, *J* = 7, H–C(3)); 0.8–2.0 (*m*, 12 H); 0.94 (*t*, *J* = 7, Me(6)); 0.82 (*s*, 2 Me–C(2′)); 0.79 (*d*, *J* = 6, Me–C(6′)). Anal. calc. for $C_{15}H_{28}O$ (224.38): C 80.29, H 12.58; found: C 80.33, H 12.54.

Fractional Crystallization of the 4-Nitrobenzoates of Racemic **6** (4 stereoisomers). Treatment of **6** (19 g, 0.085 mol) with 4-nitrobenzoyl chloride (24.1 g, 0.130 mol) in CH_2Cl_2 (250 ml) and pyridine (30 ml) gave the 4-nitrobenzoates (24.7 g, 78%). Three crystallizations from MeOH afforded the crystalline racemic 4-nitrobenzoate (10.1 g, 41%) of (±)-(1′*RS*,3*RS*,6′*RS*)-1(2,2,6-trimethylcyclohexyl)hex-1-en-3-ol. GC (corresponding acetates): 3rd and 4th peaks, t_R 25.18, 25.27, de 98% (see *Fig.*,*b*). ^{1}H-NMR: 8.26 (*m*, 4 arom. H); 5.49

(*m*, H–C(1), H–C(2), H–C(3)); 0.8–2.0 (*m*, 12 H); 0.97 (*t*, *J* = 7, Me(6)); 0.82 (*s*, Me–C(2′)); 0.80 (*s*, Me–C(2′)); 0.70 (*d*, *J* = 6, Me–C(6′)). Anal. calc. for $C_{22}H_{31}NO_4$ (373.49): C 70.75, H 8.37, N 3.75; found: C 70.78, H 8.41, N 3.71.

The mother liquors were enriched by the other racemic 4-nitrobenzoate diastereoisomer. GC (corresponding acetates): 1st and 2nd peaks; t_R 25.03, 25.10; de 56% (see *Fig. a*). The major component of this 4-nitrobenzoate mixture showed the following ^{1}H-NMR: 8.26 (*m*, 4 arom. H); 5.49 (*m*, H–C(1), H–C(2), H–C(3)); 0.80–2.0 (*m*, 12 H); 0.97 (*t*, *J* = 7, Me(6)); 0.82 (*s*, Me–C(2′)); 0.75 (*s* + *d*, *J* = 6, Me–C(2′), Me–C(6′)).

*(±)-(1′*RS,*3*RS,*6′*RS*)-1-(2,2,6-Trimethylcyclohexyl)hex-1-en-3-ol*. The crystalline 4-nitrobenzoate of (±)-(1′*RS*,3*RS*,6′*RS*)-1-(2,2,6-trimethylcyclohexyl)hex-1-en-3-ol (10 g, 0.027 mol) was hydrolysed with NaOMe (0.041 mol) in MeOH (100 ml), to afford (±)-(1′*RS*,3*RS*,6′*RS*)-1-(2,2,6-trimethylcyclohexyl)hex-1-en-3-ol (5.80 g, 96%). GC (corresponding acetates): de 98% (see above). ^{1}H-NMR: in accordance with that of racemic **6** (4-stereoisomers).

*Enzymic Acetylation of (±)-(1′*RS,*3*RS,*6′*RS*)-1-(2,2,6-Trimethylcyclohexyl)hex-1-en-3-ol*. A mixture of (±)-(1′*RS*,3*RS*,6′*RS*)-(2,2,6-trimethylcyclohexyl)hex-1-en-3-ol (10 g, 0.044 mol; de 98%), lipase PS (*Pseudomonas cepacia*; 10 g), and vinyl acetate (15 ml) in ^{t}BuOMe (100 ml) was stirred at r.t. for 24 h. After filtration and evaporation of the filtrate, the residue was submitted to CC (hexane/AcOEt 7:3). The first eluted fractions provided (1′*R*,3*R*,6′*R*)-acetate **8** (5.50 g, 47%). GC: 3rd peak; t_R 25.18; ee 98% (see *Fig. ,c*). $[\alpha]_D^{20} = +130$ (*c* = 1, $CHCl_3$). ^{1}H-NMR: 5.14–5.53 (*m*, H–C(1), H–C(2), H–C(3)); 2.06 (*s*, MeCOO); 0.8–1.8 (*m*, 12 H); 0.90 (*t*, *J* = 7, Me–C(6)); 0.81 (*s*, 2 Me–C(2′)); 0.73 (*d*, *J* = 6, Me–C(6′)). Anal. calc. for $C_{17}H_{30}O_2$ (266.42): C 76.64, H 11.35; found: C 76.60, H 11.39.

Further CC fractions yielded (1′*S*,3*S*,6′*S*)-alcohol **6a** (4.4 g, 44%). GC (corresponding acetate): 4th peak; t_R 25.27; ee 98%. $[\alpha]_D^{20} = -12$ (*c* = 1.25, $CHCl_3$). ^{1}H-NMR: consistent with that of the racemic starting material.

Absolute Configuration of Acetate **8**. A sample of **8** (1 g, 3.8 mmol) was hydrolyzed with NaOMe (5.64 mmol) in MeOH (10 ml) and then oxidized with MnO_2 (1.5 equiv.) in CH_2Cl_2 (10 ml). After evaporation, the residue was submitted to CC (hexane/AcOEt 9:1); *(1′*R,*6′*R*)-1-(2,2,6-trimethylcyclohexyl)hex-1-en-3-one* (**9**; 0.51 g, 59%). $[\alpha]_D^{20} = -29.9$ (*c* = 1, $CHCl_3$) ([11]: $[\alpha]_D^{20} = +31.5$ (*c* = 1.7, $CHCl_3$) for the (1′*S*,6′*S*)-enantiomer). ^{1}H-NMR: 6.56 (*dd*, *J* = 11, 15, H–C(1)); 6.06 (*d*, *J* = 15, H–C(2)); 2.54 (*t*, *J* = 7, CH_2(4)); 1.73 (*m*, H–C(1′)); 1.66 (*sext.*, *J* = 7, CH_2(5)); 0.8–1.55 (*m*, 7 H); 0.96 (*t*, *J* = 7, Me(6)); 0.90 (*s*, Me–C(2′)); 0.84 (*s*, Me–C(2′)); 0.77 (*d*, *J* = 7, Me–C(6′)). Anal. calc. for $C_{15}H_{26}O$ (222.37): C 81.02, H 11.78; found: C 81.07, H 11.83.

*(−)-(1′*S,*3*R,*6′*R*)-1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol* (**3c**). (1*R*′,3*R*,6′*R*)-Acetate **8** (4 g, 0.015 mol) was hydrolyzed with NaOMe (0.023 mol) in MeOH (30 ml); after the usual workup, the residue was submitted to CC (hexane/AcOEt 7:3): *(1′*R,*3*R,*6′*R*)-1-(2,2,6-trimethylcyclohexyl)hex-1-en-3-ol* (2.92 g, 87%; $[\alpha]_D^{20} = +10$ (*c* = 1, $CHCl_3$); ^{1}H-NMR: in accordance with the corresponding racemic material). This unsaturated alcohol (2.9 g, 0.013 mol) was hydrogenated in AcOH (10 ml) in the presence of PtO_2 (10% w/w), to afford, after purification, enantiomerically pure **3c** (1.88 g, 64%). $[\alpha]_D^{20} = -11.6$ (*c* = 8, EtOH) ([7]: $[\alpha]_D = -11.1$ (*c* = 9.9, EtOH). ^{1}H-NMR: 3.51 (*m*, H–C(3)); 0.8–1.61 (*m*, 15 H); 0.89 (*t*, *J* = 7, Me(6)); 0.86 (*s* + *d*, *J* = 6, Me–C(2′), Me–C(6′)); 0.76 (*s*, Me–C(2′)); 0.51 (*m*, H–C(1′)). Anal. calc. for $C_{15}H_{30}O$ (226.40): C 79.60, H 13.36; found: C 79.55, H 13.41.

*(+)-(1′*R,*3*S,*6′*S*)-1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol* (**3a**). (1′*S*,3*S*,6′*S*)-Alcohol **6a** (4.0 g, 0.018 mol) was hydrogenated in AcOH (20 ml) in the presence of PtO_2 (10% w/w), to afford, after purification, enantiomerically pure **3a** (2.44 g, 60%). $[\alpha]_D^{20} = 11.9$, (*c* = 7, EtOH) ([7]: $[\alpha]_D = +12.6$ (*c* = 10, EtOH). ^{1}H-NMR: in accordance with that of enantiomer **3c**.

*(−)-(1′*S,*3*S,*6′*R*)-1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol* (**3b**). (1′*S*,3*R*,6′*R*)-Alcohol **3c** (1.8 g, 7.96 mmol) was treated with TsCl (2.30, 11.9 mmol) in CH_2Cl_2 (20 ml) and pyridine (5 ml). After the usual workup, the tosylate derivative was submitted to acetate displacement in refluxing DMF (250 ml) in the presence of NaOAc (1.0 g, 12 mmol). The acetate derivative (1.28 g, 60%), recovered from the mixture by CC, was hydrolyzed with NaOMe (1.90 mmol) in MeOH (25 ml): enantiomerically pure **3b** (0.863 g, 80%). $[\alpha]_D^{20} = -11.2$ (*c* = 10, EtOH) ([7]: $[\alpha]_D = -11.6$ (*c* = 10, EtOH). ^{1}H-NMR: in accordance with that of diastereoisomer **3c**.

*(+)-(1′*R,*3*R,*6′*S*)-1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol* (**3d**). As described for **3b**, (1′*R*,3*S*,6′*S*)-alcohol **3a** (2.0 g, 8.85 mmol) was converted to enantiomerically pure **3d** (0.939 g, 47%). $[\alpha]_D^{20} = +11.9$ (*c* = 10, EtOH) ([7]: $[\alpha]_D = +12.1$ (*c* = 10, EtOH). ^{1}H-NMR: in accordance with that of enantiomer **3b** and of diastereoisomer **3c**.

(±)-1-(2,6,6-Trimethylcyclohex-2-enyl)hex-1-en-3-one (**4**). 8,12-Dimethyltrideca-5,7,11-trien-4-one (30.0 g, 0.14 mol) was added dropwise to 85% H_3PO_4 soln. (150 ml) at r.t. with stirring. After 40 min, the mixture was poured into ice and extracted with AcOEt. The org. layer was washed with H_2O, then with a sat. $NaHCO_3$ soln.,

dried (Na_2SO_4) and evaporated. The light yellow oil was submitted to CC (hexane/AcOEt 9:1): **4** (20.8 g, 68%). ^{1}H-NMR: 6.64 (*dd*, *J* = 10, 16, H–C(1)); 6.06 (*d*, *J* = 16, H–C(2)); 5.49 (br. *s*, H–C(3′)); 2.54 (*t*, *J* = 7.2, CH_2(4)); 2.27 (*d*, *J* = 10, H–C(1′)); 2.08 (*m*, 2 H); 1.3–1.1 (*m*, 7 H); 0.94 (*t*, *J* = 7, Me(6)); 0.92 (*s*, Me–C(6′); 0.85 (*s*, Me–C(6′)). Anal. calc. for $C_{15}H_{24}O$ (220.35): C 81.76, H 10.98; found: C 81.82, H 10.95.

1-(2,6,6-Trimethylcyclohex-2-enyl)hex-1-en-3-ol (**7**; 4 stereoisomers). Reduction of **4** (33 g, 0.15 mol) with $NaBH_4$ in MeOH at 0° gave **7** (32 g, 93%) as a 1:1 mixture of two racemic diastereoisomers. GC (corresponding acetates): 3 peaks in a 1:1:2 ratio; t_R 24.61, 24.78, 24.93. ^{1}H-NMR: 5.44 (*m*, 3 olef. H); 4.08 (*m*, H–C(3)); 1.0–2.2 (*m*, 12 H); 0.92 (*t*, *J* = 7, Me(6)); 0.88 (*s*, Me–C(2′)); 0.83, 0.81 (2*s*, Me–C(2′) of the two diastereoisomers). Anal. calc. for $C_{15}H_{26}O$ (222.37): C 81.02, H 11.78; found: C 80.97, H 11.85.

Enzymic Acetylation of Racemic **7** (4 stereoisomers). A mixture of **7** (32 g, 0.14 mol), lipase PS (*Pseudomonas cepacia*; 10 g), and vinyl acetate (48 ml) in ^{t}BuOMe (300 ml) was stirred at r.t. for 24 h. After filtration and evaporation, residue was submitted to CC (hexane/AcOEt 7:3). The first eluted fractions provided the acetates **10c**,**d** (13.9 g, 38%). GC: 1:1 mixture of the two enantiomerically pure diastereoisomers **10c** and **10d**, *i.e.* 2nd and 3rd peaks; t_R = 24.78, 24.93. ^{1}H-NMR: 5.3–5.6 (*m*, 3 olef. H); 5.20 (*m*, H–C(3)); 1.8–2.2 (*m*, 5 H); 1.0–1.8 (*m*, 10 H); 0.85–1.05 (*m*, 6 H); 0.80 (*s*, Me–C(2′)). Anal. calc. for $C_{17}H_{28}O_2$ (264.41): C 72.22, H 10.67; found: C 72.18, H 10.72.

The last eluted fractions gave the unreacted alcohol stereoisomers **7a**,**b** (16.7 g, 50%), which were acetylated with Ac_2O in pyridine prior to epoxidation. GC (acetates): 1:1 mixture; 1st and 3rd peaks; t_R 24.61, 24.93.

Epoxidation of 1-(2,6,6-Trimethylcyclohex-2-enyl)hex-1-en-3-ol Acetates: 1-(1,3,3-Trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)hex-1-en-3-ol Acetates (**11a**–**d**). In separate experiments, the 1:1 mixture of enantiomerically pure **10c**,**d** (10 g, 0.04 mol) and the 1:1 mixture of the acetate derivatives (10 g, 0.04 mol) of **7a**,**b** was epoxidized with 70% 3-chloroperbenzoic acid (14.8 g, 0.06 mol) in CH_2Cl_2 (500 ml) at 0°. Each mixture was stirred for 2 h, and then filtered, the filtrate washed first with sat. $NaHCO_3$, soln. and then with sat. $NaHSO_3$ soln., dried (Na_2SO_4) and evaporated, and the residue submitted to CC (hexane/AcOEt 7:3). Starting from **10c**,**d**, **11c** (1st eluted; 3.81 g, 34%) and **11d** (2nd eluted; 3.25 g, 29%), and starting from **7a**,**b**, **11a** (1st eluted; 3.47 g, 31%) and **11b** (2nd eluted; 3.02 g, 27%) were obtained.

Data of **11c**: $[\alpha]_D^{20} = +130$ (*c* = 1.25, $CHCl_3$). ^{1}H-NMR: 5.64 (*dd*, *J* = 10, 15, H–C(1)); 5.49 (*dd*, *J* = 7, 15, H–C(2)); 5.25 (*q*, *J* = 7, H–C(3)); 3.05 (*m*, H–C(6′)); 2.05 (*s*, MeCOO); 1.3–2.0 (*m*, 9 H); 1.24 (*s*, Me–C(1′); 0.93 (*t*, *J* = 7, Me(6)); 0.89 (*s*, Me–C(3′)); 0.73 (*s*, Me–C(3′)).

Data of **11d**: $[\alpha]_D^{20} = -38$ (*c* = 1.05, $CHCl_3$). ^{1}H-NMR: 5.68 (*dd*, *J* = 10, 15, H–C(1)); 5.42 (*dd*, *J* = 7, 15, H–C(2)); 5.24 (*q*, *J* = 7, H–C(3)); 3.05 (*m*, H–C(6′)); 2.05 (*s*, MeCOO); 1.25–2.0 (*m*, 9 H); 1.22 (*s*, Me–C(1′)); 0.93 (*t*, *J* = 7, Me(6)); 0.87 (*s*, Me–C(3′)); 0.73 (*s*, Me–C(3′)).

Data of **11a**: $[\alpha]_D^{20} = -80$ (*c* = 1.31, $CHCl_3$). ^{1}H-NMR: in accordance with that of enantiomer **11c**.

Data of **11b**: $[\alpha]_D^{20} = +19$ (*c* = 1.7, $CHCl_3$). ^{1}H-NMR: in accordance with that of enantiomer **11d**.

Deoxygenation of Diastereoisomers **11a**–**d**: *1-(2,6,6-Trimethylcyclohex-2-en-1-yl)hex-1-en-3-ol Acetates* (**10a**–**d**). In separate experiments, stereoisomers **11a**–**d** were submitted to deoxygenation according to the following procedure: To a stirred mixture of **11a**–**d** (2.0 g, 0.007 mol), AcOH (25 ml), and NaOAc (1.6 g, 0.02 mol), NaI (2.2 g, 0.015 mol) was added at r.t. After 1 h, each mixture was diluted with hexane/AcOEt 1:1 and filtered, the filtrate washed with H_2O and sat. $NaHCO_3$ soln., dried (Na_2SO_4), and evaporated, and the residue purified by CC (hexane/AcOEt 7:3) **10a**–**d**, resp.

Data of **10a**: 1.20 g (65%). GC: 1st peak; t_R 24.61; ee > 99%, de 71%. $[\alpha]_D^{20} = -165$ (*c* = 0.11, $CHCl_3$). ^{1}H-NMR: 5.52 (*dd*, *J* = 9, 15, H–C(1)); 5.40 (*m*, H–C(3′)); 5.34 (*dd*, *J* = 7, 15, H–C(2)); 5.20 (*q*, *J* = 7, H–C(3)); 2.10 (*d*, *J* = 9, H–C(1′)); 2.03 (*s*, MeCOO); 1.99 (*m*, 1 H); 1.1–1.7 (*m*, 10 H); 0.92 (*t*, *J* = 7, Me(6)); 0.88 (*s*, Me–C(6′)); 0.80 (*s*, Me–C(6′)).

Data of **10b**: 1.10 g (60%). GC: 3rd peak; t_R 24.93; ee > 99%, de 90%. $[\alpha]_D^{20} = +105$ (*c* = 0.11, $CHCl_3$). ^{1}H-NMR: 5.51 (*dd*, *J* = 9, 15, H–C(1)); 5.40 (*m*, H–C(3′)); 5.35 (*dd*, *J* = 7, 15, H–C(2)); 5.21 (*q*, *J* = 7, H–C(3)); 2.10 (*d*, *J* = 9, H–C(1′)); 2.04 (*s*, MeCOO); 2.00 (*m*, 1 H); 1.1–1.7 (*m*, 10 H); 0.93 (*t*, *J* = 7, Me(6)); 0.90 (*s*, Me–C(6′)); 0.81 (*s*, Me–C(6′)).

Data of **10c**: 1.33 g (72%). GC: 2nd peak; t_R 24.78; ee > 99%, de 91%. $[\alpha]_D^{20} = +254$ (*c* = 3.6, $CHCl_3$). ^{1}H-NMR: in accordance with that of enantiomer **10a**.

Data of **10d**: 1.26 g (68%). GC: 3rd peak; t_R 24.93 min; ee > 99%, de > 99%. $[\alpha]_D^{20} = -167$ (*c* = 2.9, $CHCl_3$). ^{1}H-NMR: in accordance with that of enantiomer **10b**.

Hydrogenation of Racemic **7** (4 stereoisomers) *and of the 1:1 Mixture* **7a**,**b**: cis- *and* trans-*1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol* (**2** and **3**, resp.). In separate experiments, racemic **7** and the 1:1 mixture **7a**,**b** in AcOH were hydrogenated in the presence of 10% Pd/C (30% w/w) at r.t. and at 3 atm. Each mixture was

filtered, the filtrate evaporated, and the residue purified by CC (hexane/AcOEt 95:5). Starting from racemic **7** (5 g, 0.023 mol), a 6:4 mixture (by ^{1}H-NMR) of **2** (*cis*)/**3** (*trans*) was obtained. The first eluted CC fractions afforded pure racemic **3** (4 stereoisomers; 1.02 g, 20%). ^{1}H-NMR: 3.51 (*m*, H–C(3)); 0.8–1.61 (*m*, 15 H); 0.89 (*t*, *J* = 7, Me(6)); 0.86 (*s* + *d*, *J* = 6, Me–C(2′), Me–C(6′)); 0.79 (*s*, Me–C(2′)); 0.51 (*m*, H–C(1′)).

Starting from **7a**,**b** (5 g, 0.023 mol), a 6:4 mixture (by ^{1}H-NMR) of the corresponding hydrogenated *cis* and *trans* stereoisomers was obtained. The first eluted CC fractions afforded a 1:1 mixture of **3a**,**b** (1.02 g, 20%) nearly devoid of optical rotation. ^{1}H-NMR: in accordance with that of racemic **3**. ^{13}C-NMR: 73.0, 72.8 (C(3)); 54.3, 54.2 (C(1′)); 42.9 (C(3′)); 40.7, 40.6, 40.3, 40.1 (C(2), C(4); not assigned); 37.3 (C(5′)); 35.30, 35.15 (C(6′)); 35.20, 35.10 (C(2′)); 31.4 (*Me*–C(2′)); 26.2 (C(1)); 22.8 (C(4′)); 21.9, 21.8 (*Me*–C(2′)); 20.72, 20.70 (*Me*–C(6′)); 19.5 (C(5)); 14.7 (C(6)).

Enzymic Acetylation of Racemic **3** (*4 stereoisomers): 1-(2,2,3-Trimethylcyclohexyl)hexan-3-ol Acetates* (**12a**–**d**). Racemic **3** (10 g, 0.044 mol), prepared as a 1:1 mixture of two racemic diastereoisomers **3a**,**c** and **3b**,**d** (GC (corresponding acetates): 4 peaks; t_R 25.82, 25.85, 25.89, 25.97) according to [5], was acetylated in ^{t}BuOMe (100 ml) in the presence of lipase PS (10 g) and vinyl acetate (20 ml). After 24 h, the mixture was filtered, the filtrate evaporated, and the residue submitted to CC (hexane/AcOEt, 8:2): **12c** (2.24 g, 19%). GC: 3rd peak; t_R 25.89; ee >99%, de 81%. $[\alpha]_D^{20} = +8.7$ (*c* = 2.7, $CHCl_3$). ^{1}H-NMR: 4.83 (*m*, H–C(3)); 2.01 (*s*, MeCOO); 0.8–1.6, (*m*, 15 H); 0.90 (*t*, *J* = 7, Me(6)); 0.87 (*s* + *d*, *J* = 6.7, Me–C(2′), Me–C(6′)); 0.77 (*s*, Me–C(2′)); 0.50 (*ddd*, *J* = 2.9, 4.8, 10.8, H–C(1′)). ^{13}C-NMR: 171.6 (COO); 75.4 (C(3)); 54.1 (C(1′)); 42.9 (C(3′)); 37.3, 37.0, 36.7 (C(5′), C(2), C(4); not assigned); 35.1 (C(2′)); 35.0 (C(6′)); 31.4 (*Me*–C(2′)); 25.6 (C(1)); 22.8 (C(4′)); 21.9 (*Me*OCO); 21.7 (*Me*–C(6′)); 20.7 (*Me*–C(2′)); 19.3 (C(5)); 14.6 (C(6)). Anal. calc. for $C_{17}H_{32}O_2$ (268.4): C 76.06, H 12.02; found: C 76.11, H 12.08.

The recovered (CC) unreacted alcohol **3a**,**b**,**d** (7.1 g, 0.03 mol), deprived of stereoisomer **3c**, was treated again with lipase PS under the same conditions. After 3 days, usual workup yielded **12d** (1.45 g, 18%). GC: 4th peak; t_R 25.97; ee >99%, de 95%. $[\alpha]_D^{20} = +21.2$ (*c* = 3.3, $CHCl_3$). ^{1}H-NMR: 4.81 (*m*, H–C(3)); 2.01 (*s*, MeCOO); 0.8–1.6 (*m*, 15 H); 0.89 (*t*, *J* = 7, Me(6)); 0.87 (*s* + *d*, *J* = 6.7, Me–C(2′); Me–C(6′)); 0.76 (*s*, Me–C(2′)); 0.49 (*ddd*, *J* = 2.6, 4.8, 10.8, H–C(1′)). ^{13}C-NMR: 171.5 (COO); 75.4 (C(3)); 54.1 (C(1′)); 42.9 (C(3′)); 37.3, 37.1, 36.9 (C(5′), C(2), C(4); not assigned); 35.2 (C(2′)); 35.1 (C(6′)); 31.3 (*Me*–C(2′)); 25.7 (C(1)); 22.8 (C(4′)); 21.9 (*Me*OCO); 21.7 (*Me*–C(6′)); 20.7 (*Me*–C(2′)); 19.3 (C(5)); 14.6 (C(6)).

*(+)-(1′*R*,3*S*,6′*S*)-1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol* (**3a**) *and (−)-(1′*S*,3*S*,6′*R*)-1-(2,2,6-Trimethylcyclohexyl)hexan-3-ol* (**3b**). In separate experiments, acetates **12c** and **12d** were hydrolyzed and converted to the corresponding alcohol **3b** ($[\alpha]_D^{20} = -8$ (*c* = 10, EtOH)) and **3a** ($[\alpha]_D^{20} = +12$ (*c* = 10, EtOH)) by acetate displacement according to the procedure described above (see **3b**).

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