Chemistry of Natural Compounds and Bioorganic Chemistry

Terpenes in organic synthesis

15.* Chemo-enzymatic approach to the synthesis of all four stereoisomers of 2,6-dimethyloct-1-yl formate, an attractant of the smaller flour beetle, from enantiomeric β-citronellenes

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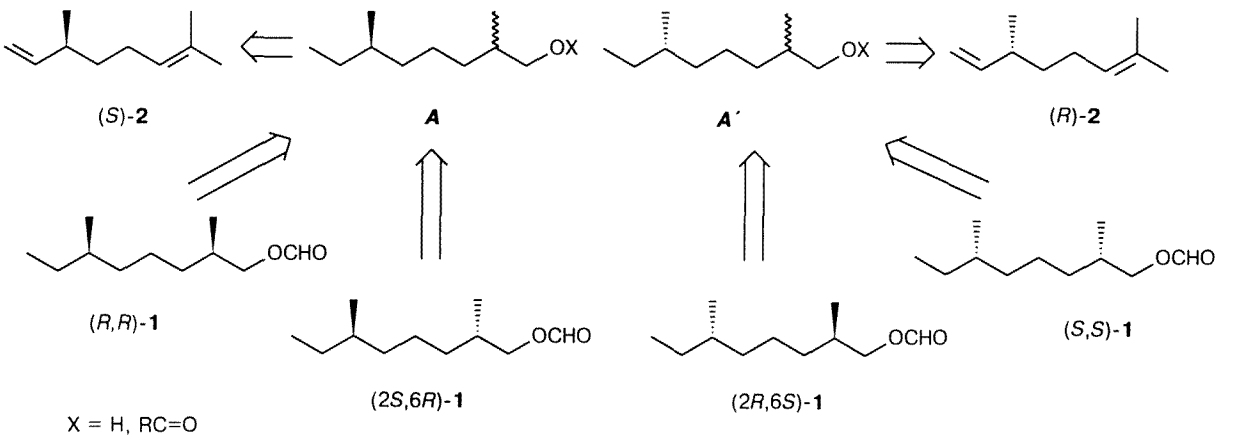
All four stereoisomers of 2,6-dimethyloctan-1-ol, the nearest precursors of the title formates, were synthesized in five to eight stages, with configurational purity ranging from 41 to 96 %, employing a stereodivergent scheme based on the partial hydrolysis of two pseudoracemic substrates, (2*RS*,6*R*)-2,6-dimethyloct-1-yl formate and (2*RS*,6*S*)-2,6-dimethyloct-1-yl acetate, in the presence of porcine pancreatic lipase (PPL). Configurations and diastereomeric compositions of the alcohols thus obtained were determined by correlating the latter with (*S*,*S*)-4,8-dimethyldecanal, prepared on the basis of enantioselective biohydrogenation of (*R*)-2,6-dimethylocta-2,7-dienal with bakers' yeast, and by comparing the $[\alpha]_D$ values of the alcohols with their NMR data and/or with those of their (*S*)-MTPA derivatives. The attractant potency of stereoisomeric 2,6-dimethyloct-1-yl formates towards *Tribolium confusum* was found to vary depending on their diastereomeric composition. The configuration at C(6) exerts some influence on the stereoselectivity of the PPL-catalyzed hydrolysis of pseudoracemic 2,6-dimethyloct-1-yl formates.

Key words: (*S*)- and (*R*)-3,7-dimethylocta-1,6-dienes; (2*RS*,6*R*)- and (2*RS*,6*S*)-2,6-dimethyloct-1-yl carboxylates, enzymatic hydrolysis; porcine pancreatic lipase; (*R*)-2,6-dimethylocta-2,7-dienal, biohydrogenation; bakers' yeast.

2,6-Dimethyloct-1-yl formate (**1**), which was originally obtained as a racemic synthetic mixture of all the four possible stereoisomers, (±)-**1**, proved to be a potent attractant for the smaller flour beetle *Tribolium confusum*,[2] whose natural aggregation pheromone is (*R*,*R*)-4,8-dimethyldecanal.[3] Subsequent syntheses[4,5] were aimed only at the racemic form of the attractant, (±)-**1**. In this communication we describe a short, stereodivergent synthesis of all four stereoisomers of **1**

* For Part 14, see Ref. 1.

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 1, pp. 204—215, January, 1996.



from enantiomerically pure specimens of (*S*)-(+)- and (*R*)-(−)-3,7-dimethylocta-2,7-diene, (*S*)-**2** and (*R*)-**2**, according to the retrosynthetic scheme above.*

Two pseudoracemic pairs of 2,6-dimethyloct-1-yl carboxylates, *A* and *A'*, were employed as the key intermediates. Each pair consisted of two epimers which differed only in the configuration of the stereogenic center C(2). It was assumed that a partial hydrolysis of the esters *A* and *A'* catalysed by an enantiodiscriminating enzyme (such as porcine pancreatic lipase, PPL) would proceed with roughly the same stereoselectivity, as the configuration of the remote asymmetric center C(6) is not likely to reverse the stereochemical outcome of the reaction. Consequently, in each pair one of the epimeric carboxylates would be hydrolyzed quicker than the other, the configurations at C(2) of the fast-reacting epimers being identical and opposite to those of the slow-reacting ones; eventually, this dichotomy would yield (*R,R*)-**1** and (2*S*,6*R*)-**1** from *A* and (2*R*,6*S*)-**1** and (*S,S*)-**1** from *A'*. The feasibility of such a kinetic resolution of pseudoracemates was already demonstrated for the microbial lipase from *Pseudomonas fluorescens*.[7] Convenient precursors of *A* and *A'*, diolefins (*S*)-**2** and (*R*)-**2** of practically 100 per cent chemical and optical purity, are commercially available.

The first version of the synthesis (Scheme 1). The oxidation of (*S*)-**2** with SeO_2 and 90 % *tert*-pentylhydroperoxide (TPHP) by a slightly modified version of an earlier procedure (*cf.* Ref. 8) afforded a 4 : 1 mixture of (*S*)-(+)-2,6-dimethylocta-2,7-dien-1-ol, (*S*)-**3**, with the respective α,β-enal, (*S*)-**4**. This mixture was reduced with $NaBH_4$ to give pure alcohol (*S*)-**3** in 72.3 % yield (in two steps). Exhaustive hydrogenation of (*S*)-**3** over platinum in MeOH afforded the pseudoracemic 1 : 1 mixture of (*R,R*)- and (2*S*,6*R*)-2,6-dimethyloctan-1-ols (**5**). On treatment with hot HCOOH according to Ref. 2 alcohol **5** gave the corresponding pseudoracemic formate **6**. In the same manner diolefin (*R*)-**2** was transformed into an analogous mixture of dienol (*R*)-**3** with dienal (*R*)-**4**, which was then processed to pure (*R*)-**3** and therefrom to the pseudoracemic mixture of (*S,S*)- and (2*R*,6*S*)-2,6-dimethyloctan-1-ols (**5'**).* Finally, this mixture was converted into the corresponding formate **6'**.

The key stage of the whole synthesis was the partial hydrolysis of formates **6** and **6'** in the presence of PPL at pH 7.0.

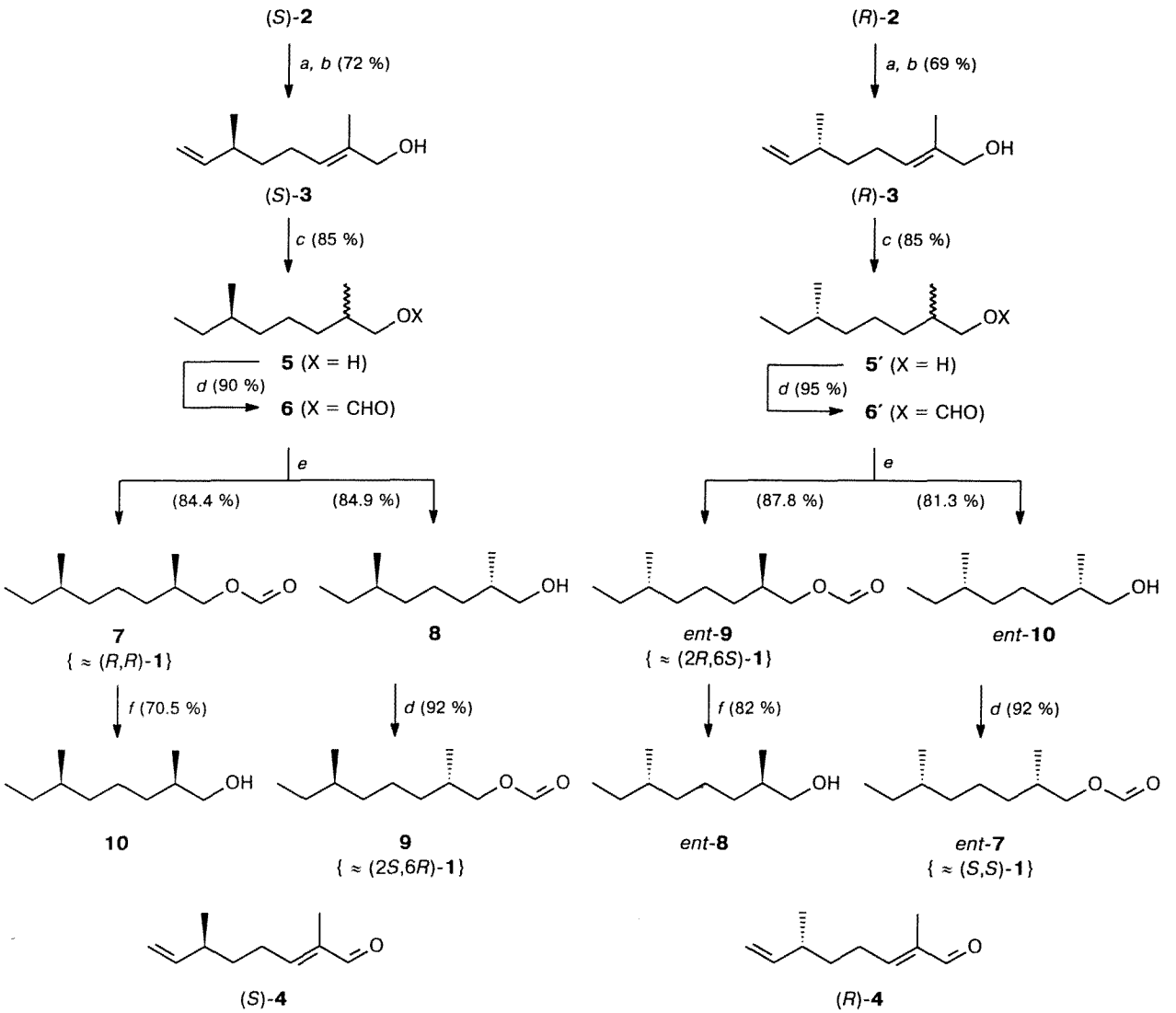
At 50 % conversion of these substrates the hydrolysis was discontinued, and its products, an alcohol and unconverted part of the formate, were separated by column chromatography on SiO_2. In this way pseudoracemic formate **6** afforded the formate **7**, enriched with (*R,R*)-diastereomer ($[\alpha]_D^{20}$ −9.21°, in hexane), and the alcohol **8**, enriched with (2*S*,6*R*)-diastereomer ($[\alpha]_D^{20}$ −9.07°, in $CHCl_3$). Alcohol **8**, on treatment with hot HCOOH, afforded the respective formate **9**, while alkaline hydrolysis of formate **7** gave alcohol **10** with $[\alpha]_D^{20}$ +0.58° (in $CHCl_3$). Similarly (see Scheme 1), partial hydrolysis of formate **6'** resulted in the dextrorotatory formate ($[\alpha]_D^{20}$ +5.45°, in hexane) which was tentatively identified as the (2*R*,6*S*)-stereoisomer, *ent*-**9**, and a dextrorotatory specimen of the stereocomplementary alcohol, *ent*-**10** (specimen **A**) with $[\alpha]_D^{20}$ +4.05° (in $CHCl_3$).**

* For preliminary communication see Ref. 6

* In the preliminary communication[6] a discrepancy between the absolute values of $[\alpha]_D$ for alkanols **5** and **5'** (−7.40° and +5.32°, respectively, in $CHCl_3$) was noted. Repetitive reproduction of all operations along the path (*R*)-**2** → (*R*)-**3** → **5'** showed that careful purification of the products formed at all stages of this transformation, as well as completeness of the exhaustive hydrogenation of (*R*)-**3** to give **5'**, provide for practically identical values of $|[\alpha]_D|$ for **5** and **5'**.

** Here and below (both in the text and on the schemes) the formates **7**, **9**, *ent*-**7**, and *ent*-**9**, which, unlike pseudoracemic formates **6** and **6'**, are binary mixtures of epimers containing (*R,R*)-**1**, (2*S*,6*R*)-**1**, (*S,S*)-**1**, and (2*R*,6*S*)-**1**, respectively, as the predominant components, are encoded independently in order to emphasize their non-identity with the latter. The prefix *ent*-, which points to enantiomeric relationships between the components of the similarly encoded binary mixtures, *does not imply* that the ratios of epimers in **7** and *ent*-**7** or in **9** and *ent*-**9** are necessarily equal.

Scheme 1



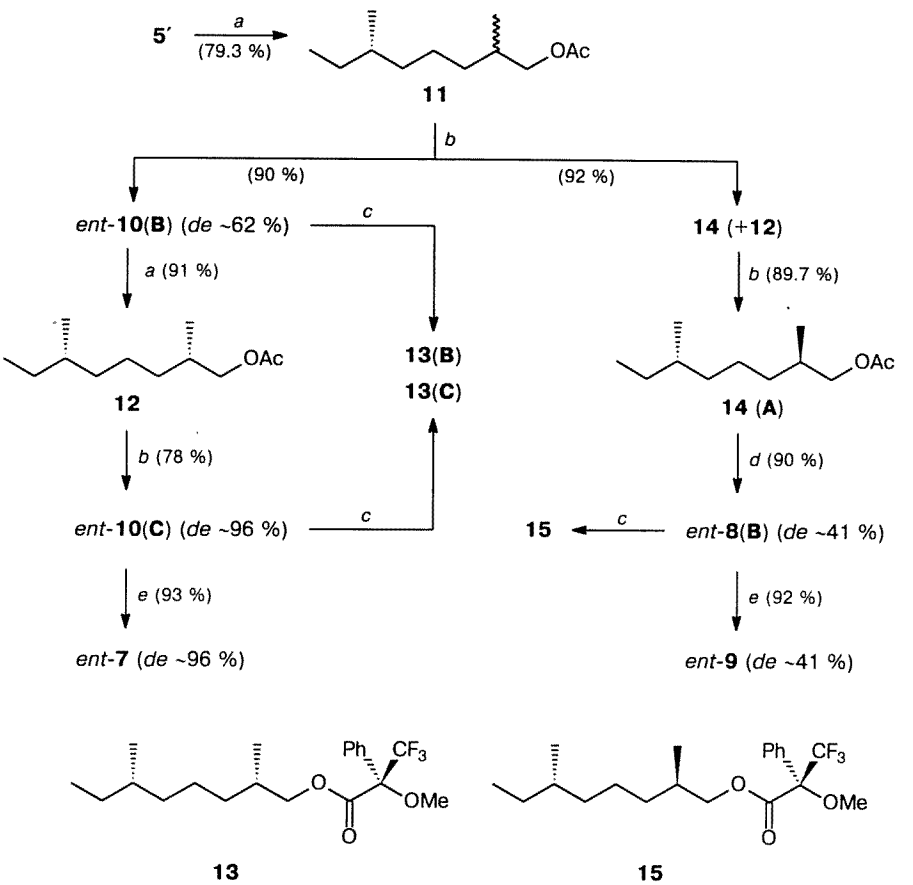
Reagents and condition: *a*. SeO_2—TPHP/CH_2Cl_2, 20 °C; *b*. $NaBH_4$/EtOH; *c*. $2H_2$—Pt/C—MeOH, 20 °C, 1 atm; *d*. HCOOH, 65 °C, 30 min; *e*. PPL/H_2O (pH 7.0), 37 °C, 50±2 % conversion; *f*. KOH—MeOH, 20 °C.

Alkaline hydrolysis of formate *ent*-**9** gave a dextrorotatory specimen of alcohol *ent*-**8(A)** with $[\alpha]_D^{20}$ +6.08° (in $CHCl_3$), whereas one of the alcohols derived from pseudoracemic formate **6** was dextrorotatory, and the other was levorotatory. This discrepancy implied that the enzymatic hydrolysis of **6′** might be less stereoselective; as the result, the liberated alcohol (tentatively, the levorotatory *ent*-**10**) would be largely contaminated with the dextrorotatory diastereomer (tentatively, *ent*-**8**) whose contribution to the specific rotation of the specimen obtained at 50 % conversion outweighs that of *ent*-**10**. Since the kinetic resolution of **6′** appeared to be inefficient (possibly, due to the high rate of enzymatic hydrolysis of alkyl formates, *cf.* Ref. 9), the respective branch of the initial synthetic plan had to be modified.

Modification of the synthetic scheme (Scheme 2). The key stage to be modified, *i.e.*, the enzymatic hydrolysis of the 2*RS*,6*S*-configurated substrate, was made more stereoselective by substituting the respective acetate (**11**) for formate **6′** and diminishing the conversion depth.

This acetate, prepared from the pseudoracemic alcohol **5′**, was hydrolyzed in presence of PPL to 35 % conversion, which resulted directly to a levorotatory specimen of alcohol *ent*-**10** with $[\alpha]_D^{20}$ −3.01° (in $CHCl_3$). Earlier,[10] for a specimen of enantiomeric (*R,R*)-2,6-dimethyloctan-1-ol of ~95 % optical purity it was reported $[\alpha]_D$ +6.9° (in $CHCl_3$); consequently, for upgrading the diastereomeric purity of *ent*-**10** it was necessary to repeat the kinetic resolution. The levorotatory *ent*-**10** (specimen **B**) was reacetylated, and the

Scheme 2



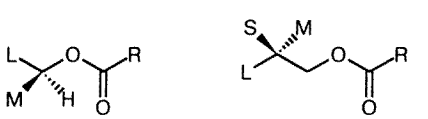
Reagents and conditions: *a.* Ac_2O—DMAP—Py, 20 °C; *b.* PPL/H_2O (pH 6.5), 37 °C, ~35 % conversion; *c.* (*R*)-MTPA-Cl—Py/CCl_4, 20 °C; *d.* KOH—MeOH, 20 °C; *e.* HCOOH, 65 °C, 30 min.

resulting acetate **12** was hydrolysed to 34 % conversion in presence of PPL to give another specimen of the levorotatory alcohol, *ent*-**10(C)**, with $[\alpha]_D$ −6.90° (in $CHCl_3$). Specimens *ent*-**10(B)** and *ent*-**10(C)** were converted to the respective esters of (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid ((*S*)-MTPA), specimens **13(B)** and **13(C)**. Analysis of their ^{19}F NMR spectra showed that the diastereomeric composition of **13(B)** and **13(C)** was 81 : 19 and ≥98 : 2, respectively.

The acetate fraction (acetate **14**), recovered from the partial enzymatic hydrolysis of acetate **11** and enriched with the (2*R*,6*S*)-diastereomer with respect to the latter, was resubmitted to the PPL-catalysed hydrolysis which was arrested at 35 % conversion. The resulted alcohol was separated by column chromatography on SiO_2 (and discarded), and the recovered part of the substrate, specimen **14(A)**, was saponified to give another specimen of alcohol *ent*-**8** with $[\alpha]_D$ +8.86° (in $CHCl_3$) which had a higher content of the predominant diastereomer (specimen *ent*-**8(B)**). It was converted to the corresponding (*S*)-MTPA ester **15** which, according to the ^{19}F NMR spectroscopy data, contained the same two diastereomers as the specimens **13(B)** and **13(C)**, but in a reversed ratio (2*R*,6*S* : 2*S*,6*S* ≈ 70 : 30).

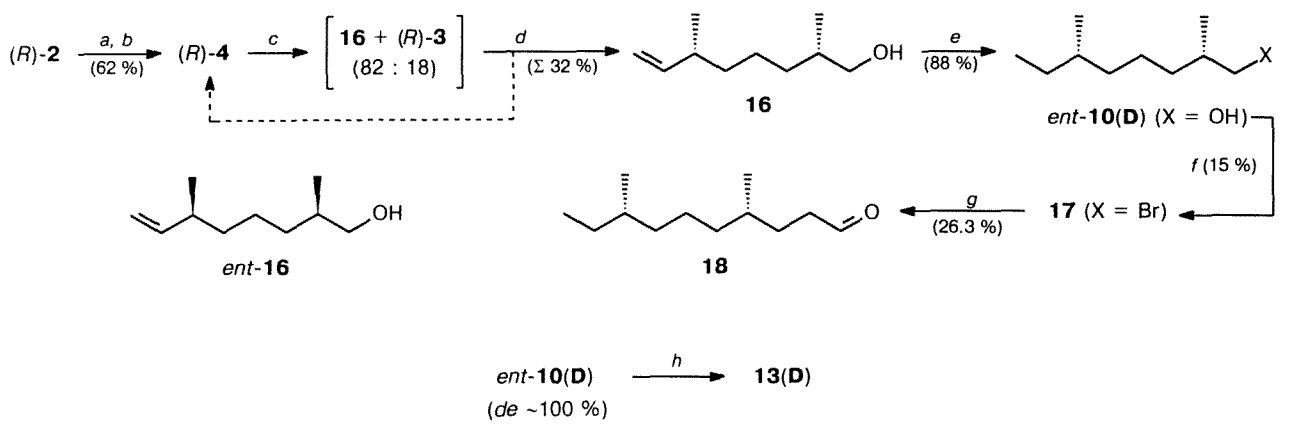
Thus, by means of three operations of partial enzymatic hydrolysis the pseudoracemic acetate **11** was converted to specimens of alcohols *ent*-**10** and *ent*-**8** of ~96 and 40 % diastereomeric purity, respectively. The formylation of *ent*-**10(C)** with hot HCOOH afforded the corresponding formate *ent*-**7**.

Configuration assignments. Synthesis of (S,S)-(+)-4,8-dimethyldecanal. Originally, the configurations of preponderant diastereomers in formates **7**, **9**, *ent*-**7** and *ent*-**9** and the respective alcohols were postulated on the basis of the well-known tendency of PPL to accelerate the hydrolysis of the esters possessing the configurations of the type ***B*** or ***C***,



B ***C***

Scheme 3



Reagents and conditions: *a.* SeO_2—TPHP/CH_2Cl_2, 20 °C; *b.* PCC—AcONa/CH_2Cl_2, 20 °C; *c.* BY/H_2O (pH 5.0—5.5), 33—35 °C, 72 h; *d.* MnO_2/hexane, 20—22 °C; *e.* H_2—Pt/C—MeOH, 20—22 °C, 1 atm; *f.* HBr (aq), Δ; *g.* (*1*) Li[CH_2=CH=NBu^t]/hexane—THF—HMPA, −70 → −50 °C; (*2*) HCl (aq), 20 °C, 1.5 h; *h.* (*R*)-MTPA-Cl—Py/CCl_4, 22 °C.

where L, M, and S denote substituents which are respectively largest, medium, and smallest in effective size; if L and M = Alk and S = Alk or H, the configuration of substrate is *S*.

However, this trend is far from being a rule, and the reasons of the observed irregularities are not always clear.[11,12] Therefore, with a view of confirming the tentative configuration assignments made for compounds **7**—**10** and *ent*-**7**—*ent*-**10**, a sterechemical correlation, relying on the well-documented[12—14] stereoselectivity of the biohydrogenation of activated trisubstituted double bonds by bakers' yeast (BY),* was undertaken (Scheme 3).

A mixture of alcohol (*R*)-**3** with aldehyde (*R*)-**4** (~4 : 1), obtained from diene (*R*)-**2** in the SeO_2—TPHP system, was oxidized with pyridinium chlorochromate (PCC) to give pure (*R*)-**4** (*cf.* Ref. 8). The enal (*R*)-**4** was incubated for 72 h at 33—35 °C and pH 5.0—5.5 with commercial fresh pressed bakers' yeast (*Saccharomyces cerevisiae*, industrial strain GOST 171-81) on a medium with D-glucose; every 24 h a half of the previously introduced amount of yeast and glucose was added. The early product of biohydrogenation was the allylic alcohol (*R*)-**3**, which further underwent a slow reduction to (2*S*,6*R*)-2,6-dimethyloct-7-en-1-ol (**16**). The concentration of the starting (*R*)-**4** in the culture medium sharply decreased at the early stage of fermentation (down to ~7 % of the original level) and then remained almost permanent for the rest of a 72 h period. This seems to be due to a rapidly established redox equilibrium between (*R*)-**3** and (*R*)-**4** under the fermentation conditions; its steady-state character possibly reflects the slowness with which the trisubsituted double bond of (*R*)-**3** is reduced.

After 72 h of fermentation the content of alkenol **16** in the medium reached its maximum; at this point the ratio **16** : (*R*)-**3** was ~82 : 18 (according to the GC and ^{1}H NMR spectroscopy data). The admixture of (*R*)-**3** in this mixture of the alcohols was selectively oxidized with active MnO_2 to give the starting enal (*R*)-**4** which was easily separated from **16** by chromatography on silica gel. The sign of specific rotation of alcohol **16** ($[\alpha]_D^{22}$ −15.5°, in $CHCl_3$) was opposite to that found earlier[15] for (2*R*,6*S*)-2,6-dimethyloct-7-en-1-ol (*ent*-**16**). The yield of **16** from (*R*)-**4** was 32 % overall, which is equivalent to a 19—20 % yield from the starting diolefin (*R*)-**2**.

Catalytic hydrogenation of compound **16** afforded a saturated alcohol with $[\alpha]_D$ −7.32° (in $CHCl_3$). As followed from its GC and ^{13}C NMR spectroscopy data, this alcohol was an individual substance which practically coincided with specimen *ent*-**10(C)**, obtained from acetate **11** by two consequtive operations of PPL-catalyzed partial hydrolysis. This alcohol, *ent*-**10(D)**, on treatment with α-methoxy-α-(trifluoromethyl)phenylacetyl chloride prepared from (*S*)-MTPA afforded a chemically pure (*S*)-MTPA ester, **13(D)**, which displayed only one singlet at 70.74 ppm in its ^{19}F NMR spectrum. The same signal dominates in the ^{19}F NMR spectra of specimens **13(B)** and **13(C)** whereas in the spectrum of (*S*)-MTPA ester **15** it appears as the minor one. The identity of *ent*-**10(D)** with the major component of specimens *ent*-**10(B)** and *ent*-**10(C)** proves that the yeast reduction of aldehyde (*R*)-**4**, just as in the case

* Earlier it was shown that the biohydrogenation of all the isoprenoidal[13] and non-isoprenoidal[12,14] α,β-enals and/or respective allylic alcohols of the general type (*E*)-RCH=C(Me)CHO and/or (*E*)-RCH=C(Me)CH_2OH by fermenting bakers' yeast uniformly gave rise to *S*-configurated alkanols of the general type $RCH_2CH(Me)CH_2OH$.

of other substrates of this structural type, gives rise to a product with *S*-configuration of the resulting asymmetric center C(2). Consequently, the PPL-catalyzed hydrolysis of pseudoracemic carboxylates **6'** and **11** leads preponderantly to the *S*-configurated alcohol. In other words, characteristic stereoselectivity of the PPL-catalyzed hydrolysis is retained in this particular case.

Still, there remained some doubt about the reliability of the *S*-configuration assigned to the C(2) atom in alcohols **16** and *ent*-**10**. This was due to a discrepancy between the absolute values of $[\alpha]_D$ found for our specimen of alcohol **16** and for its antipode, *ent*-**16**, which was synthesized earlier[15] from two chiral building blocks of ~95—100 % optical purity (−15.5° and +6.79°, respectively, in $CHCl_3$). Therefore, for an unambigous proof of the configuration of **16**, and hence of *ent*-**10(D)**, the latter was heated with hydrobromic acid to give the corresponding bromide **17**, which was then made to react with ethylidene-*N*-*tert*-butylamine under the conditions of Stork's *C*-alkylation (see Scheme 3). The resulting product was hydrolyzed with diluted HCl and purified by column chromatography, which afforded (*S,S*)-(+)-4,8-dimethyldecanal (**18**) with $[\alpha]_D^{20}$ +7.22° in 26.3 % yield. This specimen coincided with the previously described aldehyde[16,17] in its IR and ^{1}H NMR spectra. Comparing the $[\alpha]_D$ of **18** found in this work with $[\alpha]_D^{22}$ values reported earlier[16,17] for specimens of 98—100 % optical purity (+7.3° to +7.33°, in $CHCl_3$) shows that the optical purity of aldehyde **18** prepared from alcohol **16** is ~97 %.

The above stereochemical correlation is equivalent to a synthesis of aldehyde **18** from enal (*R*)-**4** in 3.5 % overall yield; this corresponds to *ca.* 2.1 % yield based on diolefin (*R*)-**2** (over six steps, without optimization). Although aldehyde **18** is devoid of attractant activity towards *Tribolium confusum* and *T. castaneum* flour beetles, there is a possibility of its being a part of the aggregation pheromone of a closely related species, *T. freemani*.[17,18]

Diastereoselectivity of enzymatic hydrolysis of 2,6-dimethyloct-1-yl carboxylates. Unlike the enzymatic optical resolution of racemic esters, PPL-catalyzed hydrolysis of pseudoracemic carboxylates **6**, **6'**, and **11** results in the binary mixtures of products with identical configuration at C(6), but opposite configurations at C(2). In principle, the composition of such mixtures can be determined quantitatively by gas chromatography on a column with an achiral stationary phase.

However, employing the packed columns under standard conditions of GC it was not possible to obtain good peak resolution for the epimeric alcohols. Therefore, diastereomer composition of their binary mixtures (and that of the corresponding 2,6-dimethyloct-1-yl carboxylates) was determined polarimetrically using the equation

$$[\alpha]_D(\text{obs}) = [\alpha_1]_D \cdot (1-x) + [\alpha_2]_D \cdot x, \qquad (1)$$

where $[\alpha]_D$(obs) is specific rotation of a given pair of epimers, $[\alpha_1]_D$ and $[\alpha_2]_D$ are individual specific rotations of the major and minor component of this pair (all recorded in the same solvent), and x is the molar part of the minor component.*

On the basis of the data obtained for specimen *ent*-**10(D)** (*vide supra*) the value of $[\alpha]_D^{20}$ for the 100 % pure (*S,S*)-2,6-dimethyloctan-1-ol was assumed to be −7.32°. Hence, for its pure *R,R*-antipode $[\alpha]_D$ must be +7.32° (in $CHCl_3$). For a specimen of (2*R*,6*S*)-2,6-dimethyloctan-1-ol (*ent*-**8**), obtained earlier[10] by a route providing for ~100 % enantiomeric purity of both asymmetric centers, it was reported that $[\alpha]_D$ is +15.7° (in $CHCl_3$). Therefore, the $[\alpha]_D$ of its antipode, alcohol **8**, should be −15.7°. These four values are in a qualitative agreement with the superposition of the $[\alpha]_D$ values found for (*R*)- and (*S*)-6-methyloctan-1-ol (−8±0.1° and +7.9±0.1°, respectively, in $CHCl_3$)[19] and for (*R*)- and (*S*)-2-methyloctan-1-ol (+11.2° and −11.2° respectively, in CH_2Cl_2).[20]

Substituting the above $[\alpha]_D$ values of pure alcohol **8**, *ent*-**8**, **10**, and *ent*-**10** into Eq. (1) gave the ratios of the C(2)-epimers in their binary mixtures of various origin (Table 1).

Similar results were obtained when the diastereomeric ratios in the heterochiral dimethyloctanols with 6*S*-configuration were determined by ^{19}F NMR spectroscopy (for the respective specimens of MTPA esters **13** or **15**) and ^{13}C NMR spectroscopy (for free alcohols *ent*-**8** and *ent*-**10**). The percent composition of binary mixtures calculated by Eq. (1) almost coincided with that calculated from the ratios of integral intensitites of characteristic signals in the NMR spectra (*cf.* Tables 1 and 2).

The data in Table 1 can be summarized as follows.

1. Stereoselectivity of PPL-catalyzed partial hydrolysis of formate **6** and, particularly, formate **6'** is rather modest. At 50 % conversion, the diastereomeric purity of the resultant alcohol is slightly above 40 % in the former case and close to zero in the latter.

2. Enzymatic hydrolysis of pseudoracemic acetate **11** occurs with a higher diastereoselectivity than that of the respective formate **6'**.

3. The PPL-catalyzed hydrolysis of carboxylates **6**, **6'**, and **11** preponderantly affects diastereomers with a 2*S*-configuration.

Apparently, the stereodivergent synthesis of formates (*R,R*)-**1**, (2*S*,6*R*)-**1**, (2*R*,6*S*)-**1**, and (*S,S*)-**1** from diolefins (*S*)-**2** and (*R*)-**2** could be optimized by employing other, more deastereospecific lipases instead of PPL.

When employing PPL, overall yields of the four target formates and their diastereomeric purity (assumed to correspond to that of the respective alcohols) were the following: for (*R,R*)-**1** (specimen **7**) — 23.2 % over

* The diastereomeric composition of the unreacted formates or acetates recovered from the partial enzymatic hydrolysis was determined from that of the respective alcohols obtained upon saponifying these esters; the asymmetric center at C(2) was assumed not to be affected under the saponification conditions.

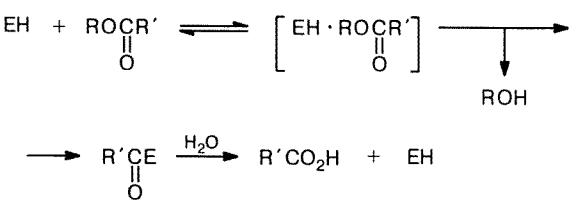
Table 1. Specific rotations and diastereomeric compositions of the binary mixtures of stereoisomeric 2,6-dimethyloctan-1-ols, resulting from partial PPL-catalyzed hydrolysis of diastereomeric 2,6-dimethyloct-1-yl carboxylates or from saponification of the unconverted ester fractions recovered after enzymatic hydrolysis

Alcohols (configuration)	Origin and marking of specimens	Conversion[a] (%)	$[\alpha]_D$ ($CHCl_3$)	Diastereomeric composition (%)[b]		*de*[b] (%)
				major isomer	minor isomer	
ent-**10** (*S,S*)	PPL-catalyzed hydrolysis of formate **6'** (specimen **A**)	50±2 %	+4.05°	50.6 (53)	49.4 (47)	1.2 (≤6)
	PPL-catalyzed hydrolysis of acetate **11** (specimen **B**)	35±2 %	−3.01°	81.3 (81)	18.7 (19)	62.6 (62)
	Second hydrolysis of unconverted acetate (specimen **C**)	35±2 %	−6.90	98.2 (≥98)	1.8 (≤2)	96.4 (≥96)
10 (*R,R*)	Saponification of formate **9**[c]	[c]	+0.58°	70.7	29.3	41.4
ent-**8** (2*R*,6*S*)	Saponification of formate *ent*-**9**[c] (specimen **A**)	[c]	+6.08°	58.2 (57—59)	41.8 (41—43)	16.4 (14—18)
	Saponification of acetate **14** recovered after repeated PPL-catalyzed hydrolysis of acetate **11** (specimen **B**)[d]	[d]	+8.86°	70.3 (70.0)	29.7 (30.0)	41.0 (40.0)
8 (2*S*,6*R*)	PPL-catalyzed hydrolysis of formate **6**	50±2 %	−9.07°	71.2	28.8	42.4

[a] Determined from GC and ^{1}H NMR spectroscopy data of the global neutral fraction of the hydrolysis products. [b] Calculated from Eq. (1) by assuming the absolute values of $[\alpha]_D$ to be |7.32°| for (*R,R*)-**1** and (*S,S*)-**1** (that is, for diastereomerically homogeneous specimens of alcohols **10** and *ent*-**10**) and |15.7°| for (2*S*,6*R*)-**1** and (2*R*,6*S*)-**1** (that is, for diastereomerically homogeneous specimens of alcohols **8** and *ent*-**8**). The bracketed figures relate to the data obtained from ^{13}C NMR spectra of free alcohols and/or ^{19}F NMR spectra of (*S*)-MTPA esters **13** and **15** prepared from *ent*-**10** and *ent*-**8**, respectively. [c] The formates taken for saponification corresponded to 50±2 % conversion of the pseudoracemic precursors (**6** or **6'**) upon enzymatic hydrolysis. [d] The acetate used in this saponification was recovered after two successive operations of enzymatic hydrolysis (each time to ~35 % conversion) of pseudoracemic acetate **11**.

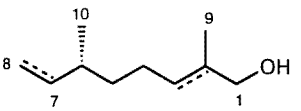
five steps starting from (*S*)-**2**, *de* ~41 %; for (2*S*,6*R*)-**1** (specimen **9**) — 21.5 % over six steps starting from (*S*)-**2**, *de* ~42 %; for (*S,S*)-**1** (specimen *ent*-**7**(**C**)) — 3.3 % over eight steps starting from (*R*)-**2** (Scheme 2), *de* ~96.4 %; for (2*R*,6*S*)-**1** (specimen *ent*-**9**(**B**)) — 11.1 % over eight steps starting from (*R*)-**2**, *de* ~41 %.

A marked difference in the stereoselectivity of enzymatic hydrolysis, observed for pseudoracemic formates **6** and **6'**, is to be noted. In the diastereomer pair **6**, component (*R,R*)-**1** is hydrolyzed slower that its counterpart (2*S*,6*R*)-**1**. On the other hand, both components of **6'**, (*S,S*)-**1** and (2*R*,6*S*)-**1**, are hydrolyzed at nearly the same rate. Considering the current mechanism of the ester hydrolysis assisted by serine-containing hydrolases,[21] which comprises the formation of a molecular complex of the enzyme (EH) with an ester, subsequent transformation of this complex (EH · ROCOR') into a covalently acylated enzyme (ECOR'), and concomitant liberation of an alcohol molecule ("pushed out" by a hydroxy group in the active site of the enzyme),



the difference in the reactivity of **6** and **6'** may be attributed to the effect of the remote methyl group at C(6) either on the rate of complexing or on the feasibility of the transesterification reaction in the inner sphere of the complex. Since both diastereomers in the formate **6'** are hydrolyzed at comparable rates, the first assumption seems to be more plausible. In such a case, the difference in the hydrolysis diastereoselectivity, observed for pseudoracemates **6** and **6'**, might be explained in terms of a *conformational substrate model* which would take into account the ability of PPL to anchor predominantly that of the two competing substrates which fits better the transition state geometry in the active site.

Table 2. ^{13}C NMR spectra of (*R*)-(−)-2,6-dimethylocta-2,7-dien-1-ol ((*R*)-**3**) and of the homo- and heterochiral products of its transformation



Compound, specimen	δ (in $CDCl_3$)[a]					Diastereomeric ratio[b]
	C(8)	C(9)	C(10)	C(1)	others	
(*R*)-**3**	112.79	13.26	20.26	69.10	126.45 (C(3)); 134.74 (C(2)); 144.58 (C(7))	~100 : 0
(*R*)-**4**	113.63	9.29	20.35	195.41	139.43 (C(3)); 154.87 (C(2)); 143.73 (C(7))	~100 : 0
16	112.45	16.62	20.32	68.43	144.89 (C(7))	~100 : 0
ent-**10(D)**	11.407	19.234	16.651	68.326	—	~100 : 0
ent-**10(A)**	11.407	19.232 ≤ 19.234	16.651 (0.52) 16.658 (0.48)	68.326 (0.53) 68.376 (0.47)	—	≤53 : 47
5′	11.407	19.232 ≈ 19.234	16.651 (0.52) 16.658 (0.50)	68.326 (0.49) 68.376 (0.51)	—	~50 : 50
ent-**8(A)**	11.408	19.232 ≥ 19.234	16.651 (0.41) 16.658 (0.59)	68.326 (0.43) 68.376 (0.57)	— —	~42 : 58

[a] For heterochiral compounds figures in the brackets indicate relative integral intensity of the corresponding signals (a mean of 3—4 runs at 25 °C and 2—3 runs at 45 °C). [b] For **5′** and *ent*-**10(A)** the differences in the intensity of identical signals are within the mean error of measuring their peak areas. No signals attributable to the *Z*-isomers of (*R*)-**3** and (*R*)-**4** were detected in their spectra.

Attractant potency as a function of configuration and diastereomeric purity. Olfactometric study of the attractant activity of formates **7**, **9**, *ent*-**7**, and *ent*-**9** towards the mixed (sex/age) populations of the smaller flour beetle, *Tribolium confusum*, revealed the dependence of the attractant potency of tested specimens on their diastereomer composition.*

Formate **7**, which contained *ca.* 71 % of (*R,R*)-**1**, and formate *ent*-**9(A, B)**, which contained *ca.* 58 or 70 % of (2*R*,6*S*)-**1**, at a dose of 10 μg per dispenser attracted up to 60 % of the insects in the test group. These results are roughly comparable with the attractant potency of the standard racemic mixture of all four stereoisomers of 4,8-dimethyldecanal (about 80 % catch at the same dose). Pseudoracemate **6**, which contained just 50 % of (*R,R*)-**1**, was almost as attractive as **7** and *ent*-**9**. On the other hand, formate **9** containing the same components as **6**, but in a different proportion (only 29 % of (*R,R*)-**1**) was substantially less attractive, and formate *ent*-**7(B)** containing ~81 % of (*S,S*)-**1** displayed only marginal activity comparable with that of the control.

The results of the bioassays are reminiscent of earlier observations made upon testing the reception of the four stereoisomers of 4,8-dimethyldecanal by *Tribolium confusum* and *T. castaneum*.[3,22] In these tests the (*R,R*)-stereoisomer (natural aggregation pheromone) and (4*R*,8*S*)-stereoisomer (a pheromone synergist) were attractive to both species, whereas their 4*S*-antipodes were not. Since the former two aldehydes are isosteric with formates (*R,R*)-**1** and (2*R*,6*S*)-**1**, the parallelism of their attractant activity implies the importance of chiral recognition for pheromone reception of *T. confusum*. However, by applying the efficacy-to-cost criterion to the above pheromone mimics one may recommend use of pseudoracemic formate **6** as the attractant of choice, since its yield from (*S*)-**2** amounts to 55 % over the four stages of synthesis.

* The bioassays were conducted in 1993—1994 by V. N. Burov, E. A. Shavrina and A. P. De Millo (Institute of Plant Protection, Russian Academy of Agricultural Sciences, St. Petersburg), to whom the authors express their gratitude. A detailed account of these studies will be published elsewhere.

Experimental

^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker WM-250 spectrometer; ^{19}F NMR spectra were also run in $CDCl_3$ (using $CFCl_3$ as the external reference) on a Bruker AC-200P instrument (188.3 MHz). IR spectra were taken in $CHCl_3$ on a UR-20 spectrometer (Carl Zeiss, Jena). Gas chromatography was performed on an LKhM-8 MD instrument provided with a flame ionization detector and a 1.5×0.003 m stainless steel column packed with 5 % SE-30 on Chromaton N-AW-DMCS, using N_2 (60 mL min^{-1}) as the carrier gas; the oven temperature: 120 °C (for the C_{10} alkanols and their formates), 130 °C (for olefins **3**, **4**, and **16** and aldehyde **18**), and 135 °C (for alkanols and their acetates). TLC analyses were done using Silufol plates with a fixed silica gel coating. Column chromatography was carried out on silica gel L (40—100 μm, Czech Republic). Specific rotation was measured on a JASCO-DIP 360 polarimeter.

Starting diolefins (*S*)-**2** and (*R*)-**2** with $[\alpha]_D$ +9.7° and −9.5°, respectively, and (*S*)-(−)-MTPA (Fluka AG, Switzerland) were used without additional purification. Active manganese dioxide was prepared according to Ref. 23. Porcine pancreatic lipase (47.8 U mg^{-1}) was purchased from Biolar, Latvia. Commercial fresh pressed bakers' yeast (GOST 171-81, produce of the Moscow Yeast Factory, Mosagroprom) was checked for cell integrity and the absence of microbial infestation.

(2*E*,6*S*)-(+)-2,6-Dimethylocta-2,7-dien-1-ol ((*S*)-3). To a stirred suspension of SeO_2 (315 mg, 2.83 mmol) in CH_2Cl_2 (22 mL) 25 mL of 90 % TPHP (195 mmol) and subsequently diene (*S*)-**2** (7.5 g, 54.3 mmol) were added. The reaction mixture was stirred for 7 h at 20—22 °C, then diluted with benzene (60 mL), and concentrated under reduced pressure to remove CH_2Cl_2. The remainder was dissolved in Et_2O (60 mL), and the organic phase was thoroughly washed with 10 % aqueous KOH (4×15 mL) and water (20 mL), dried with Na_2SO_4, and evaporated *in vacuo*. Analysis by gas chromatography (130 °C) showed the residue to be an 80 : 20 mixture of alcohol (*S*)-**3** (R_t 7.0 min) and aldehyde (*S*)-**4** (R_t 6.0 min).

This material was added to a stirred suspension of $NaBH_4$ (190 mg, 5 mmol) in EtOH (5.5 mL), the reaction mass was agitated for 4 h at 20—22 °C, then left for 15 h, and quenched with 5 % aqueous HCl (10 mL). The reaction product was extracted with Et_2O (50 mL, with stirring), the ethereal layer was separated, washed with water, and dried ($MgSO_4$). The extract was concentrated *in vacuo*, and the residue was chromatographed on a column of Al_2O_3 (activity grade II) using a hexane—Et_2O gradient (0 → 100 % Et_2O, v/v). Pure dienol (*S*)-**3** was isolated as a colorless oil with b.p. 124 °C (45 Torr) and $[\alpha]_D^{22}$ +7.22° (*c* 1.55, $CHCl_3$). Yield: 6.05 g (72.3 %). IR, ν/cm^{-1}: 3610, 3450, 1640, 995, 920. ^1H NMR, δ: 1.05 (d, 3 H, 6-Me, J = 6 Hz); 1.20—1.40 (m, 2 H, 5-H_2); 1.65 (d, 3 H, 2-Me, J = 1 Hz); 1.95—2.10 (m, 3 H, 4-H_2 and 6-H); 3.95 (s, 2 H, C$\underline{H}_2$OH); 4.65 (br.s, 1 H, OH); 4.93 (dd, 1 H); 4.98 (dd, 1 H); 5.70 (ddd, 1 H, J_{AC} = 18 Hz, J_{BC} = 9.5 Hz, $J_{H(6),H(7)}$ = 7.0 Hz, ABC-system, 8-H_2 and 7-H); 5.38 (m, 1 H, 3-H).

(2*E*,6*R*)-(−)-2,6-Dimethylocta-2,7-dien-1-ol ((*R*)-3) was obtained from (*R*)-**2** (7.5 g) by the same procedure. Colorless oil with b.p. 124 °C (45 Torr), R_t 7.0 min, $[\alpha]_D^{22}$ −7.18° (*c* 1.32, $CHCl_3$). Yield: 69.6 %. ^1H NMR spectrum was identical with that of (*S*)-**3**. For the ^{13}C NMR spectrum see Table 2.

(2*RS*,6*R*)-(−)-2,6-Dimethyloctan-1-ol (5). A solution of dienol (*S*)-**3** (1.1 g, 7.1 mmol) in methanol (6 mL) and 0.65 g of the catalyst (5 % Pt on charcoal, w/w) were placed in a stoppered flask, provided with a magnetic stirrer and connected with a gas holder, and vigorously agitated under the atmosphere of H_2 (20—22 °C, 1 atm); 320 mL of H_2 (14.2 mmol) was consumed in 34 h. The catalyst was removed by filtration and washed with three portions of MeOH. The combined methanolic solution was evaporated under reduced pressure, and the residue (1.0 g) was chromatographed on a column of Al_2O_3 (20 mL) using a hexane—Et_2O gradient (1 → 50 % Et_2O, v/v) as the eluent to afford the alkanol **5** as a viscous colorless oil with R_t 7.5 min and $[\alpha]_D^{20}$ −7.40° (*c* 2.16, $CHCl_3$). Yield: 0.97 g (85 %). IR, ν/cm^{-1}: 3350, 1365, 1040. ^1H NMR, δ: 0.85 (t, J = 7 Hz) + 0.91 (d) + 1.01 (d) (overlapping signals of 9 H, 8-H_3, 6-Me, and 2-Me); 1.05—1.60 (m, 10 H, 4-H_2, 5-H_2, 7-H_2, 3-H_2, 6-H, 2-H); 2.03 (br.s, 1 H, OH); 3.45 (m, 2 H, C$\underline{H}_2$OH).

(2*RS*,6*S*)-(+)-2,6-Dimethyloctan-1-ol (5′) was obtained from dienol (*R*)-**3** (1.08 g) using exactly the same procedure. Yield: 0.95 g (85.3 %). Colorless oil with R_t 7.5 min and $[\alpha]_D^{20}$ +7.32° (*c* 2.33, $CHCl_3$). The IR and ^1H NMR spectra of **5′** fully coincided with those of alkanol **5**. For the ^{13}C NMR spectrum see Table 2.

Substituting palladium catalysts (5 and 10 % Pd on charcoal, w/w) for the platinum one substantially accelerated the hydrogenation, but this was accompanied with the formation of hydrogenolysis products.

(2*RS*,6*R*)-(−)-2,6-Dimethyloct-1-yl formate (6) was obtained using an earlier procedure[2] by heating alkanol **5** (0.95 g) with freshly distilled HCOOH (10 mL) at 65 °C for 30 min. The reaction mixture was neutralized at 0—5 °C with a saturated solution of $NaHCO_3$, and the product was extracted with Et_2O at pH 8.0. Column chromatography on SiO_2 (20 g) in a hexane—Et_2O gradient (99 → 49 % hexane) afforded the formate **6** as a colorless oil with R_t 9.3 min and $[\alpha]_D^{20}$ −8.62° (*c* 1.02, hexane). Yield: 1.0 g (90 %). IR, ν/cm^{-1}: 1725 and 1180 (Alk—O—CHO). ^1H NMR, δ: 0.85 (t, 3 H, 8-H_3, J = 6 Hz); 0.94 and 0.96 (two overlapping doublets of 6 H, 6-Me and 2-Me, J = 7 Hz); 1.03—1.55 (m, 9 H, 4-H_2, 5-H_2, 7-H_2, 3-H_2, and 6-H); 1.80 (m, 1 H, 2-H); 4.0 (d, 2 H, 1-H_2, J = 6.5 Hz); 8.1 (s, 1 H, OCH=O).

(2*RS*,6*S*)-(+)-2,6-Dimethyloct-1-yl formate (6′) was prepared from alkanol **5′** (0.80 g) exactly as above. Colorless oil, R_t 9.3 min, $[\alpha]_D^{20}$ +8.49° (*c* 1.41, hexane). Yield: 0.90 g (95.6 %). IR and ^1H NMR spectra of **6′** practically coincided with those of the formate **6**.

Enzymatic hydrolysis of formate 6. Sterilized 0.1 *M* phosphate buffer (pH 7.0, 0.3 mL) and formate **6** (300 mg, 1.6 mmol) were placed into a round bottom flask equipped with a magnetic stirring rod, and the substrate was dispersed in the buffer by vigorus stirring. Dry powdered PPL (160 mg, *i.e.*, 100 mg of PPL per mmol of substrate) was added to the emulsion. The flask was stoppered, and the stiring was continued at 37 °C for about 20 h; the pH was kept at 7.0 by regular addition of 1 *N* aqueous NaOH. Aliquots of the reaction mass were periodically taken, and the progress of the hydrolysis was followed by titrating the liberated HCOOH and by measuring the substrate/product ratio by gas chromatography. At 50±2 % conversion the reaction mixture was saturated with NaCl and extrated with Et_2O (5×5 mL). The combined organic layer was washed with water, dried ($MgSO_4$), and concentrated *in vacuo*. The residue (0.25 g) was chromatographed on a column of silica gel (5 g) using a hexane—Et_2O gradient (1 → 50 % Et_2O, v/v). The early, less polar fractions afforded the unreacted part of the substrate, formate **7**, as a colorless oil with R_t 9.3 min and $[\alpha]_D^{20}$ −9.21° (*c* 1.91, hexane). Yield: 127 mg (84.4 %). IR and ^1H NMR spectra of formate **7** were practically undistinguishable from those of the starting formate **6**.

From more polar fractions alcohol **8** was isolated as a colorless oil with R_t 7.5 min and $[\alpha]_D^{20}$ −9.07° (*c* 1.10, $CHCl_3$). Yield: 108 mg (84.9 %). IR and ^1H spectra of alcohol **8** almost coincided with those of the pseudoracemic alcohol **5**.

Enzymatic hydrolysis of formate 6′. The above procedure was applied to formate **6′** (300 mg, 1.6 mmol). At 50 % conversion it gave the formate *ent*-**9**, an oil with R_t 9.3 min and $[\alpha]_D^{20}$ +5.45° (*c* 1.43, hexane), and alcohol *ent*-**10** (specimen **A**), an oil with R_t 7.5 min and $[\alpha]_D^{20}$ +4.05° (*c* 1.20, $CHCl_3$). The yields of *ent*-**2** and *ent*-**10(A)** were 132 mg (87.8 %) and 111 mg (87.3 %), respectively. The data of IR and ^1H NMR spectra for compounds *ent*-**9** and *ent*-**10(A)** were largely analgous to those of **6′** and **5′**. For the ^{13}C NMR spectrum of *ent*-**10(A)** see Table 2.

Saponification of formates 7 and *ent*-9 (general procedure). To a solution of powdered KOH (45 mg, 0.81 mmol) in MeOH (1.5 mL) 100 mg of formate **7** (0.54 mmol) was

added. The reaction mass was stirred at 20—22 °C for 2.5 h to complete the saponification of **7** (TLC monitoring, development with hexane—Et_2O, 5 : 1). Then the reaction mixture was concentrated under reduced pressure to one third of its volume, the remainder was diluted with water (2 mL), and the product was extracted with Et_2O (4×5 mL). The extract was evaporated to leave a residue (0.7 g), which was chromatographed on a column of Al_2O_3 (14 g) using a hexane—Et_2O gradient. Elution with 50 : 50 (v/v) hexane—Et_2O afforded alcohol **10** as a colorless oil with R_t 7.5 min and $[\alpha]_D^{20}$ +0.58° (*c* 2.48, $CHCl_3$). Yield: 60 mg (70.5 %). IR and ^1H NMR spectra of alcohol **10** display the same absorption bands and signals as those of pseudoracemic alcohol **5**.

In the same maner formate *ent*-**9** was saponified to give alcohol *ent*-**8** (specimen **A**), a colorless oil with R_t 7.5 min and $[\alpha]_D^{20}$ +6.08° (*c* 3.50, $CHCl_3$); its IR and ^1H NMR spectra were almost indistiguishable from those of alcohols **5** and **5'**. Yield: 90 mg (82 %). For the ^{13}C NMR spectrum of *ent*-**8(A)** see Table 2.

(2*RS*,6*S*)-(+)-2,6-Dimethyloct-1-yl acetate (11). To a solution of alcohol **5'** (1.0 g, 6.3 mmol) in dry pyridine (1.2 mL) a solution of freshly distilled acetic anhydride (0.96 g, 9.3 mmol) and 4-dimethylaminopyridine (30 mg, ~0.25 mmol) in pyridine (0.2 mL) was added. This mixture was stirred at 22 °C for 4 h, then neutralized with 0.1 *M* HCl, and extracted with Et_2O (5×10 mL). The extract was washed with saturated solutions of $NaHCO_3$ and NaCl, dried with $MgSO_4$, and evaporated. The residue (1.1 g) was chromatographed on a column of silica gel (20 g) using a hexane—Et_2O gradient (99 → 49 hexane, v/v) to afford acetate **11** as a colorless oil with R_t 9.1 min (130 °C) and $[\alpha]_D^{20}$ +7.07° (*c* 1.42, hexane). Yield: 1.0 g (79.3 %). IR, ν/cm^{-1}: 1730, 1245 (Alk—O—COMe). ^1H NMR, δ: 0.85 (d, 3 H, 6-Me, J = 6.5 Hz); 0.88 (t, 3 H, 8-H_3, J = 6.5 Hz); 0.93 (d, 3 H, 2-Me, J = 7 Hz); 1.0—1.4 (m, 9 H, 4-H_2, 5-H_2, 7-H_2, 3-H_2, 6-H); 1.65 (m, 1 H, 2-H); 2.05 (s, 3 H, O=CMe); 3.95 (m, 2 H, 1-H_2).

Enzymatic hydrolysis of acetate 11. ***Stage 1.*** To a stirred suspension of PPL (0.5 g) in 3 mL of phosphate buffer (pH 6.5) acetate **11** (1.0 g, 5 mmol) was added at 20 °C and dispersed in the medium by vigorous stirring (5 min). The tempeature was raised to 37 °C, and hydrolysis was carried out in the same manner as described above for formates **6** and **6'**; the pH was kept at 6.5 by gradual neutralization of the liberated AcOH with 1 *N* aqueous solution of NaOH. When 35 % conversion was attained, the reaction mass was saturated with NaCl and extracted with Et_2O (5×15 mL). The combined extract was washed with water, dried ($MgSO_4$), and concentrated in a rotary evaporator. The remainder (0.9 g) was chromatographed on a column of SiO_2 (25 mL) using a hexane—Et_2O gradient (10 → 50 % Et_2O, v/v). First there was eluted 0.60 g of unconverted substrate (a mixture of acetates **14** and **12** in which the former predominated, yield 92 %) followed by alcohol *ent*-**10** (specimen **B**), a colorless oil with R_t 7.0 min (135 °C) and $[\alpha]_D^{22}$ −3.01° (*c* 1.0, $CHCl_3$), which was identified from its IR spectrum. Yield: 0.25 g (90 %). The ^{19}F NMR spectrum of (*S*)-MTPA derivative **13** prepared from *ent*-**10(B)** (*vide infra*) revealed that it consisted of two diastereomers in a ratio of 81 : 19 (*cf.* Table 2).

Stage 2. The above alcohol *ent*-**10(B)** (237 mg, 1.5 mmol) was acetylated with Ac_2O (200 mg, 1.9 mmol) and DMAP (20 mg) in dry pyridine (1.5 mL) for 3 h at 20—22 °C. The reaction was worked-up as described above for the preparation of acetate **11** to give the acetate of corresponding diastereomeric purity (**12**) as an oil with $[\alpha]_D^{22}$ +4.41° (*c* 0.8, $CHCl_3$). Yield: 273 mg (91 %).

Acetate **12** (273 mg) was subjected once again to enzymatic hydrolysis at 37 °C and pH 6.5 in presence of PPL (140 mg) in phosphate buffer (0.3 mL). After 18 h of exposure, when the conversion attained ~34 %, the reaction mixture was worked-up as described above for the hydrolysis of acetate **11**. Column chromatography on SiO_2 (10 g) resulted in alcohol *ent*-**10** (specimen **C**) of high diastereomeric purity, $[\alpha]_D^{22}$ −6.90° (*c* 1.0, $CHCl_3$). Yield: 50 mg (78 %). IR, ν/cm^{-1}: 3610, 3350, 1365, 1040. ^1H NMR spectrum of *ent*-**10(C)** was almost undistinguishable from that of the starting alcohol **5'**.

Stage 3. The acetate fraction (**14** > **12**) recovered from the enzymatic hydrolysis of acetate **11** at stage 1 (0.6 g, 3 mmol) was resubjected to hydrolysis at 37 °C and pH 6.5 in presence of PPL (300 mg) dispersed in 0.6 mL of 0.1 *M* phosphate buffer. At 35 % conversion the reaction was arrested, and the reaction mass was worked-up as described above for the hydrolysis of acetate **11**. The neutral products were column chromatographed on SiO_2 using a hexane—Et_2O gradient (10 → 50 % Et_2O) to afford a diastereomerically enriched specimen of (2*R*,6*S*)-configurated acetate **14**, as a colorless oil with $[\alpha]_D^{20}$ +3.01° (*c* 1.0, hexane). Its IR and ^1H NMR spectra were practically identical with those of pseudoracemic acetate **11**. Yield: 0.35 g (89.7 %).

Saponification of acetate 14. To a soluton of KOH (0.13 g, 2.3 mmol) in MeOH (5 mL) the above specimen of acetate **14** (0.35 g, 1.75 mmol) was added, and the solution was stirred for 2.5 h at 20—22 °C until no trace of **14** could be detected (GC and TLC control). The reaction mass was concentrated in a rotary evaporator to one third of its volume, the remainder was diluted with water (2 mL) and extracted with Et_2O (10 mL + 2×3 mL). The combined ethereal extract was washed with water, dried ($MgSO_4$), and evaporated to leave a colorless oil with R_t 7.0 min and $[\alpha]_D^{20}$ +8.86° (*c* 1.0, $CHCl_3$), which proved to be chromatografically pure (GC, TLC) alcohol *ent*-**8** (specimen **B**). Yield: 0.252 g (90 %). IR and ^1H spectra of *ent*-**8(B)** coincided with those of specimen *ent*-**8(A)**. For the ^{13}C NMR spectrum see Table 2. The (*S*)-MTPA ester of alcohol *ent*-**8(B)** (compound **15**, *vide infra*) displayed in its ^{19}F NMR spectrum two peaks, related to the (2*R*,6*S*)- and (2*S*,6*S*)-diastereomers, in a proportion of 70 : 30.

(2*E*,6*R*)-(−)-2,6-Dimethylocta-2,7-dienal ((*R*)-4). To an energetically stirred suspension of pyridinium chlorochromate (3.24 g, 15 mmol) and anhydrous sodium acetate (1.64 g, 20 mmol) in dry CH_2Cl_2 (20 mL) the 4 : 1 mixture of alcohol (*R*)-**3** with aldehyde (*R*)-**4** (1.58 g, ~8 mmol of the alcohol) was added in one portion at 20—22 °C. After six hours of stirring the reaction mixture was diluted with anhydrous Et_2O (40 mL) and left to stay for additional 6 h. The supernatant was decanted from the black tar, which was washed with dry Et_2O (3×10 mL) until it turned into a solid dispersion. The washings were combined with the supernatant, the solvents were stripped off through a small Vigreux column, and the residue (1.2 g) was chromatographed on a column of SiO_2 (25 g). Elution with hexane—Et_2O (90 : 10, v/v) gave pure aldehyde (*R*)-**4** as a colorless oil, R_t 6.0 min (130 °C), $[\alpha]_D^{20}$ −6.19° (*c* 1.70, $CHCl_3$). Yield: 0.98 g (62 %, *i.e.*, ~77.5 % based on the content of (*R*)-**3** in the starting mixture). IR, ν/cm^{-1}: 2715, 1695, 1645, 990, 915. ^1H NMR, δ: 1.03 (d, 3 H, 6-Me, J = 6.5 Hz); 1.35—1.65 (m, 2 H, 5-H_2); 1.69 (d, 3 H, 2-Me, J = 1.0 Hz); 1.95—2.45 (m, 3 H, 4-H_2, 6-H); 4.96 (dd, 1 H, 8-H_A, J_{AB} = 17.5 Hz, J_{AC} = 1 Hz); 4.98 (dd, 1 H, 8-H_B, J_{AB} = 17.5 Hz, J_{BC} = 9 Hz); 5.70 (ddd, 1 H, 7-H, J_{AC} = 1 Hz, J_{BC} = 9 Hz, $J_{H(6),H(7)}$ = 7 Hz); 6.42 (m, 1 H, 3-H); 9.43 (s, 1 H, 1-H). For the ^{13}C NMR spectrum see Table 2.

(2*S*,6*R*)-(−)-2,6-Dimethyloct-7-en-1-ol (16). Sterilized tap water (0.5 L) was placed into a round-bottom flask equipped with a mechanical stirrer, a bubbler, and a reflux condenser; then fresh pressed bakers' yeast (46.6 g) and D-glucose (23.3 g) were introduced in small portions at 35 °C while gently agitating, and the mixture was stirred for 3—3.5 h at 35 °C until foaming began. At this moment saturated solution of $NaHCO_3$ was added to the culture for adjusting the pH to 5.5, followed by a solution of the enal (*R*)-**4** (1.4 g) in 4 mL of EtOH. Then aeration was started by passing a slow stream of sterilized, filtered air (25 mL min^{-1}) through the stirred suspension of yeast cells. The onset of the fermentation was marked by a raise of the pH, so 1 *M* HCl was periodically added dropwise to the culture broth to maintain pH within 5.0—5.5. The course of the yeast reduction was monitored by GC and TLC. After 1.5 h of fermentation the content of the starting (*R*)-**4** in the culture medium fell to ~7 % of its original level, and from this moment on it practically did not change until harvesting. The first reduction product detected in the culture medium was the allylic dienol (*R*)-**3** (R_t 7.0 min at 130 °C). Its subsequent bioconversion into monoolefinic alcohol **16** (R_t 6.7 min, 130 °C) occurred at a slower pace. After 24 h of fermenting additional portions of yeast (23.3 g) and glucosee (11.5 g) were added to the reaction mass, and 24 h later this was followed by the addition of yet another portion of yeast (11.7 g) and glucose (6 g). By the third day of fermentation a steady-state equilibrium between these reduction products was attained at a ratio **16** : (*R*)-**3** = 82 : 18; further addition of yeast and glucose did not affect either this ratio, nor the content of these alcohols in the culture. The broth from the flask was filtered through a pad of gauze, and the filtrate (a fine cell suspension) was extracted with Et_2O (5×0.3 L) in a two-neck round-bottom flask provided with a mechanical stirrer.

Each time, the extracts were carefully decanted from the aqueous cell suspension, and the combined organic layer was concentrated to about one tenth of its volume and treated (while stirring) with warm 10 % aqueous NaOH (3×25 mL). The phases were separated, the alkaline aqueous phase was reextracted with Et_2O (25 mL), and the combined organic phase was washed with water, dried ($MgSO_4$), and evaporated to the permanent weight of the residue. In this way 0.85 g of a mixture of alcohol **16** and (*R*)-**3** was obtained. Analysis by gas chromatography, as well as by ^1H NMR spectroscopy (which revealed the presence of a $-CH=C(Me)CH_2OH$ grouping with its characteristic signals at δ 1.75 (s) and 5.4 (m)), showed it to contain ~20 % of allylic alcohol (*R*)-**3** and the trace amounts of enal (*R*)-**4**.

This mixture (0.85 g, ~1.1 mmol based on the content of (*R*)-**3**) was added to a vigorously stirred suspension of freshly prepared "alkaline" MnO_2 (0.87 g, 10 mmol) in dry hexane (20 mL), and the reaction mass was stirred for 72 h at 20—22 °C to complete the conversion of (*R*)-**3** into aldehyde (*R*)-**4** (GC, TLC). Inorganic solids were removed by filtration and thoroughly washed with Et_2O (10×5 mL). The filtrate and washings were combined and evaporated to give 0.75 g of a mixture of alcohol **16** with enal (*R*)-**4**, which was chromatographed on a column of SiO_2 (25 g) in a hexane—Et_2O gradient (10 → 50 % Et_2O, v/v). The early fractions were shown to contain (*R*)-**4**, while the evaporation of more polar fractions afforded pure alkenol **16** as a colorless oil with R_t 6.7 min (130 °C) and $[\alpha]_D^{22}$ −15.5° (*c* 1.16, $CHCl_3$). Yield: 0.450 g (32 %, based on the starting enal). IR, ν/cm^{-1}: 3590, 1645, 1370, 920. ^1H NMR, δ: 0.92 (d, 3 H, 2-Me, *J* = 7 Hz); 1.03 (d, 3 H, 6-Me, *J* = 7 Hz); 1.20—1.50 (m, 6 H, 4-H_2, 5-H_2, 3-H_2); 1.60 (m, 1 H, 2-H); 2.10 (m, 1 H, 6-H); 3.50 (m, 2 H, 1-H_2); 4.90 and 4.94 (m, 1 H, 8-H_2); 5.2 (br.s, 1 H, OH); 5.71 (ddd, 1 H, 7-H, J_{AM} = 15 Hz, J_{BM} = 10 Hz, $J_{H(6),H(7)}$ = 7 Hz). For the ^{13}C NMR spectrum see Table 2.

Enantiomerically pure (*S*,*S*)-2,6-dimethyloctan-1-ol (*ent*-10(D)). A solution of alcohol **16** (0.45 g) in 5 mL of methanol was stirred at 22 °C under an atmosphere of H_2 (1 atm) with 0.3 g of a platinum catalyst (5 % Pt on charcoal, w/w) until the consumption of H_2 (2 mol. equiv.) ceased, which took 35 h. The catalyst was removed by filtration and washed with Et_2O (5×5 mL), and the combined filtrates were evaporated *in vacuo* to give pure *ent*-**10** (specimen **D**), a colorless oil with R_t 7.0 min (135 °C) or 7.5 min (120 °C) and $[\alpha]_D^{20}$ −7.32° (*c* 1.68, $CHCl_3$). Yield: 0.40 g (87.9 %). The IR and ^1H NMR spectra of *ent*-**10(D)** were practically the same as those of specimens **5'** and *ent*-**10(C)**. For the ^{13}C NMR spectrum see Table 2.

When being prepared from *ent*-**10(D)**, the corresponding specimen of (*S*)-MTPA ester **13** (*vide infra*) displays only one signal in its ^{19}F NMR spectrum, namely, a singlet with δ ^{19}F 70.7. The same singlet largely prevailed in the ^{19}F NMR spectrum of the specimen prepared from *ent*-**10(C)**.

(*S*,*S*)-1-Bromo-2,6-dimethyloctane (17). A mixture of *ent*-**10(D)** (0.38 g) and 48 % hydrobromic acid (5 mL) was refluxed for 7 h. The reaction mixture was extracted with Et_2O (3×5 mL), the extract was washed with saturated aqueous $NaHCO_3$ and water to a neutral reaction, then with saturated aqueous $Na_2S_2O_3$, and again with water, dried with $MgSO_4$, and evaporated under reduced pressure. The oily residue, contaminated with chromatographically mobile hydrocarbons (GC and TLC data), was chromatographed on a column of SiO_2 (12 g) using a hexane—Et_2O gradient (0 → 10 % Et_2O). The bromide **17**, a dense colorless oil, was isolated from more polar fractions of the eluate. Yield: 86 mg (15 %). ^1H NMR, δ: 0.83 (d, 3 H, 6-Me, *J* = 6 Hz); 0.87 (t, 3 H, 8-H_3, *J* = 7 Hz); 0.92 (d, 3 H, 2-Me, *J* = 6 Hz); 1.05—1.45 (m, 9 H, 4-H_2, 5-H_2, 7-H_2, 3-H_2, 6-H); 1.60 (m, 1 H, 2-H); 3.55 (m, 2 H, 1-H_2).

(*S*,*S*)-(+)-4,8-Dimethyldecanal (18). To a vigorously stirred solution of LDA (0.36 mmol, prepared from 0.46 mL of Pr^i_2NH and 0.1 mL of 2.4 *M* solution of Bu^nLi in hexane) in a mixture of hexane (3 mL) with HMPA (0.57 mL) a solution of acetaldehyde *tert*-butylimine[24] (33 mg, 0.33 mmol) in dry THF (3 mL) was added slowly at 0 °C under the atmosphere of argon, and the stirring at this temperature was continued for additional 20 min. Then the reaction mixture was cooled to −70 °C and treated with bromide **17** (70 mg, 0.31 mmol) while stirring, after which the temperature was raised to −50 °C, and the stirring was continued for additional four hours. The reaction mass was quenched with ice-cold water, left to thaw, and extracted with Et_2O (3×10 mL). The extract was concentrated to the volume of 5 mL and mixed with 1 *M* HCl (2.5 mL). Without separating the phases, this mixture was stirred for 1.5 h to complete the neutralization of the liberated amine. Then the aqueous layer was removed, the ethereal layer was diluted with additional 10 mL of Et_2O, the organic solution was washed with saturated aqueous $NaHCO_3$ and water, and dried (Na_2SO_4). The solvent was evaporated through a small Vigreux column to leave a residue, which was chromatographed on a column of SiO_2 (2 g) using a hexane—Et_2O gradient (10 → 50 % Et_2O). In this manner chromatographically pure aldehyde **18** was isolated as a colorless oil with R_t 7.5 min (135 °C) and $[\alpha]_D^{20}$ +7.22° (*c* 1.0, $CHCl_3$). Yield: 15 mg (26.3 %). IR, ν/cm^{-1}: 2720, 1725 (—CHO). ^1H NMR, δ: 0.65—0.98 (m, 9 H, 8-Me, 10-H_3, 2-Me); 1.05—1.95 (m, 12 H, 5 CH_2, 4-H, 8-H); 2.29 (m, 2 H, 2-H_2); 8.9 (t,

1 H, CHO, J = 1.8 Hz). Lit.: $[\alpha]_D^{20}$ +7.33° ($CHCl_3$)[16] and +7.3° ($CHCl_3$).[17]

(*S*)-MTPA esters of (6*S*)-2,6-dimethyloctan-1-ols (13 and 15) (general procedure). A solution of the (*R*)-chloroanhydride, prepared from (*S*)-MTPA (0.15 mmol) according to a known procedure[25], in dry pyridine (0.15 mL) was added to a solution of an alcohol to be analyzed, *i.e.*, **5'**, *ent*-**10**, *ent*-**8** (~16 mg, 0.1 mmol) in dry CCl_4 (30 μL). After 15 min of exposure at 20—22 °C, the mixture was treated with 2-dimethylaminoetylamine[26] (0.1 mmol) dissolved in as little dry CCl_4 as possible, and, 15 min later, with 1 *M* HCl (2 mL). The phases were separated, and acidic aqueous layer was extracted with Et_2O (3×1 mL). The combined organic layer was successively washed with 1 *M* HCl, saturated aqueous $NaHCO_3$, and water, dried (Na_2SO_4), and evaporated. The residue was a chromatographically pure specimen of an (*S*)-MTPA ester, **13** or **15**, whose diastereomeric composition was determined using the integral intensity ratios of the singlets of the CF_3 groups, observed in their ^{19}F NMR spectra at δ 70.74 and 70.77 ppm; the accuracy of mesuring δ ^{19}F was within ±0.01.

The specimens of the (*S*)-MTPA esters thus obtained had the following integral intensity ratios of the singlets present in the ^{19}F NMR spectra (δ 70.74 : δ 70.77).

Ester 13 (derivatized specimens of *ent*-**10**): from specimen **B** — 81 : 19; from specimen **C** — ≥98 : 2; from specimen **D** — 100 : 0.

Ester 15 (derivatized specimen *ent*-**8(B)**) — 30 : 70.

Esters 13 + 15 (derivatized pseudoracemate **5'**) — ~50 : 50.

Diastereomerically enriched formates, *ent*-**7** (*de* ~96 %) and *ent*-**9** (*de* ~40 %), were obtained from specimens *ent*-**10(C)** and *ent*-**8(B)** in 93 and 92 % yields, respectively, in exactly the same manner as was described above for formates **6** and **6'**. All of them had practically identical IR spectra and identical values of R_t and R_f.

The authors express their gratitude to M. I. Struchkova and A. V. Ignatenko for high-precision recording of the ^{13}C and ^{19}F NMR spectra, respectively, as well as to N. E. Voishvillo for helpful suggestions regarding the microbiological part of the work.

This work was carried out with the financial support from the Russian Foundation for Basic Research (Project No. 93-03-5893).

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Received June 16, 1995