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Chiral Juvenoids Derived from 2-Substituted Cyclohexanols

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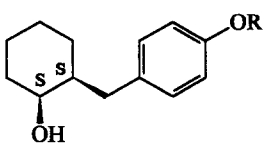
Abstract: Chiral juvenoids **1d - 4d** were prepared by a chemoenzymatic process consisting in employing microorganism-mediated biotransformations and/or enzyme-mediated transformation of convenient substrates, followed by a chemical transformation of the chiral intermediates into the chiral compounds targeted. Biological activity of the juvenoid stereoisomers **1d - 4d** on the yellow mealworm (*Tenebrio molitor*) pupae was studied.

Introduction

Henrick *et al.*[1] presented considerable differences in biological activity observed with the stereoisomers of compounds imitating the action of natural juvenile hormones (JH), implying that a chiral receptor system (and possibly more than one such a site) is involved in the insect JH response. It is supposed, however, that the interaction of juvenoids (insect juvenile hormone bioanalogs) with the receptor site(s)[2] decides in favour of one of the stereoisomers only under the assumption that the stereogenic center of the juvenoid molecule takes a direct part in interacting with the receptor active site(s),[2] or it is very close to the part of the molecule, which does.



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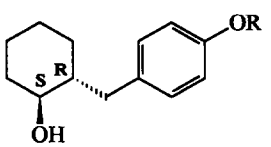

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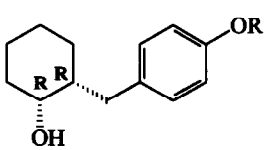

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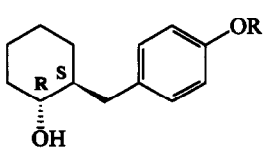

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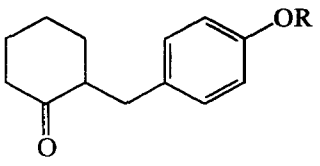
a: R=H, **b**: R=THP, **c**: R=MOM, **d**: R=$(CH_2)_2NHCOOEt$

A target of the work presented was to synthesize chiral juvenoids **1d** - **4d** using a chemoenzymatic approach consisting of employing an enzyme-mediated transformation or a microorganism-mediated biotransformation followed by a chemical transformation of suitable substrates, and to study potential differences in the biological activity of the stereoisomers **1d** - **4d** when tested on the yellow mealworm (*Tenebrio molitor*). Results obtained in this investigation could assist in augmentation of the present knowledge of understanding the role of the saturated ring of these compounds in the interaction with the receptor site(s). Wimmer *et al.*[3] prepared carbamate juvenoids **1d** - **4d** in their racemic forms and found remarkable biological activity on a broad spectrum of insect pests.[3,4]



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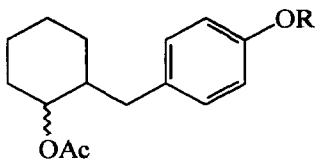


6 - *cis* racemic
7 - *trans* racemic

b: R=THP, **c**: R=MOM

In a recent paper,[5] we published a preparation of the chiral precursors **1a** - **4a** using an enzyme-mediated transformation of the THP protected substrates **5b** - **7b**. However, these substrates did not prove to be suitable enough because of certain difficulties occurred during HPLC analysis of the diastereoisomeric MTPA derivatives of **1b** - **4b**.[5] It is supposed that removal of the THP group occurred on the silica gel used as stationary phase in the HPLC columns.[5] This finding represented a difficulty in the ee (enantiomeric excess) determination. Moreover, the chemical yield of a PPL (porcine pancreatic lipase) mediated hydrolysis of **6b** and **7b** presented in the recent paper[5] was very low (cf. Table 1). Therefore, a search for a more stable protecting group was initiated. Moreover, a more suitable method of access to (1*R*,2*R*) and (1*R*,2*S*) stereoisomers **2** and **4** has also been focused on. The method employed finally consisted in the hydrolysis of suitable substrates by a *Geotrichum candidum* lipase mediated process affording the desired stereoisomers **2** and **4** with a considerable yield (Table 1). The biocatalyst (*Geotrichum candidum*) was selected on the basis of a broad screening.[6]

Results and Discussion

The unsuitable THP protecting group was substituted by MOM, which - among other advantages - shows even higher stability on silica gel[7]. The substrates **5c** - **7c** have been used for the biotransformation. Preparation of the substrates **5c** - **7c** (Scheme 1) started from 2-(4-hydroxybenzyl)- -1-cyclohexanone[8]. The sodium salt of this compound was treated with chloromethyl methyl ether[9] in benzene yielding the racemic substrate **5c**. The

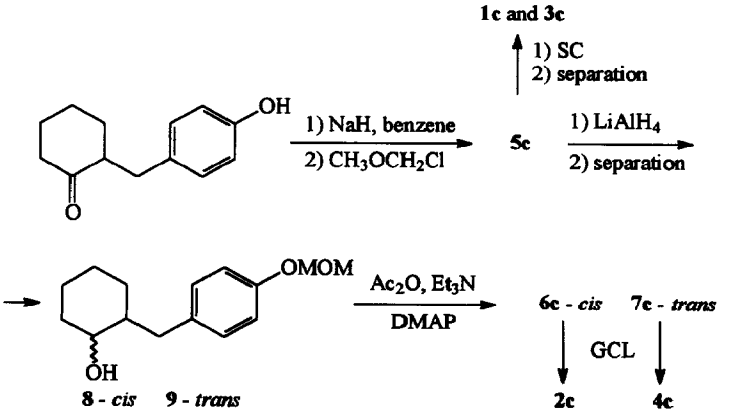
ketone **5c** was reduced by $LiAlH_4$ and a mixture of corresponding isomeric alcohols **8** and **9** was separated on silica gel. Acetylation[5] of the respective isomeric alcohols **8** or **9** yielded the racemic substrates **6c** or **7c** (Scheme 1).

An enzymatic reduction of the substrate **5c** in aqueous media (Scheme 1, Table 2) using *Saccharomyces cerevisiae*[5,10] yielded a mixture of diastereoisomeric alcohols **1c** and **3c**, separable by column chromatography on silica gel. The chemical yield of the enzymatic reaction was improved by dissolving **5c** with a small amount of acetone. A *Geotrichum candidum* lipase mediated hydrolysis of **6c** and **7c** in aqueous media (Scheme 1, Table 2) yielded the respective stereoisomers **2c** or **4c**.

Table 1. Biotransformation of the substrates **5 - 7**

Substrate	Biocatalyst	Product	AC	Yield (%)[a]	ee (%)
5b	SC[b]	**1b**	1*S*,2*S*	50	94
		3b	1*S*,2*R*	24.8	87
6b	PPL[c]	**2b**	1*R*,2*R*	5.2	85
7b	PPL[c]	**4b**	1*R*,2*S*	6.2	92
5c	SC[b]	**1c**	1*S*,2*S*	48.3	97
		3c	1*S*,2*R*	45.2	96
6c	GCL[d]	**2c**	1*R*,2*R*	33.3	98
7c	GCL[d]	**4c**	1*R*,2*S*	24.3	98

[a] theoretical yield is 50 %, [b] *Saccharomyces cerevisiae*, [c] porcine pancreatic lipase, [d] lipase from *Geotrichum candidum*



Scheme 1

The NMR assignment of the absolute configuration requires a synthesis of a pair of diastereoisomeric compounds.[11] (*S*)-(+)- and (*R*)-(-)-2-methoxy-2-phenyl-3,3,3-trifluoropropanoyl chloride (MTPA chloride)[12-15] were used for this purpose. Using the ^{1}H NMR spectra, the absolute configuration of the stereogenic center bearing a hydroxy group of the compound studied can be assigned on the basis of the difference of the chemical shifts of H-C(7) and H'-C(7) signals in the pair of the diastereoisomeric derivatives (i.e. the (*R*)- and/or (*S*)-MTPA-based esters of the alcoholic compounds studied; cf. Table 3). Evaluation of the ^{19}F NMR spectra resulted in the same assignment of the absolute configuration based on the difference in the chemical shifts of the trifluoromethyl group in the (*R*)- and (*S*)-MTPA esters derived from **1c - 4c** (Table 3). A detailed description of the assignment of absolute configuration of the stereogenic centers of compounds **1 - 4** has been recently published.[5]

Table 2. Conditions of the biotransformation reactions

Biocatalyst	*Saccharomyces cerevisiae* (5 g), strain CCY 21-4-63-e	lipase from *Geotrichum candidum* (10 mg), Amano
Cultivation[10]	48 h at 27±1°C in a liquid malt	-
Biotransformation	7 d at 27±1°C, phosphate buffer pH 7.0 (100 ml)	7 d at room temperature, phosphate buffer pH 7.0 (4.8 ml)
Substrate	**5c**, 100 mg (0.40 mmol) in 0.2 ml of acetone	**6c**, **7c**; 100 mg (0.34 mmol) in 0.2 ml of acetone

Table 3. The ^{1}H and ^{19}F NMR based assignment of the absolute configuration of **1c - 4c** and **1d - 4d** based on the analysis of their diastereoisomeric MTPA esters

Source acid	(*R*)-MTPA		(*S*)-MTPA		(*R*)-MTPA	(*S*)-MTPA	AC
Source alcohol	δ[H-C(7)]	δ[H'-C(7)]	δ[H-C(7)]	δ[H'-C(7)]	δ(CF_3)		
1c	2.24	2.45	2.33	2.51	-71.26[a]	-71.38[a]	1*S*,2*S*
2c	2.33	2.51	2.24	2.45	-71.38[a]	-71.26[a]	1*R*,2*R*
3c	2.08	2.7	2.18	2.89	-71.59[a]	-71.68[a]	1*S*,2*R*
4c	2.18	2.89	2.08	2.7	-71.68[a]	-71.59[a]	1*R*,2*S*
1d	2.25	2.45	2.33	2.5	-67.13	-67.29	1*S*,2*S*
2d	2.33	2.52	2.25	2.45	-67.31	-67.12	1*R*,2*R*
3d	2.07	2.7	2.17	2.87	-67.44	-67.54	1*R*,2*S*
4d	2.17	2.89	2.07	2.69	-67.55	-67.46	1*S*,2*R*

[a] $CFCl_3$ (δ = 0.0 ppm) was used as internal reference

Table 4. HPLC analysis of the MTPA esters derived from the alcohols **1c - 4c**

Source alcohol	AC	MTPA	HPLC area (%)	time (min)	ee (%)
		Saccharomyces cerevisiae			
1c:2c	1*S*,2*S* : 1*R*,2*R*	*R*	98.28 : 1.72	39.09 and 44.08	97
3c:4c	1*S*,2*R* : 1*R*,2*S*	*R*	98.02 : 1.98	21.39 and 24.12	96
		Lipase from *Geotrichum candidum*			
1c:2c	1*S*,2*S* : 1*R*,2*R*	*R*	0.90 : 99.10	40.07 and 43.12	98
3c:4c	1*S*,2*R* : 1*R*,2*S*	*R*	0.85 : 99.15	22.18 and 24.13	98

The enantiomeric excess of the alcohols **1c - 4c** was determined on the basis of the HPLC data of the corresponding MTPA esters (Tables 1 and 4). The MOM protecting group proved to be stable under the conditions given in the Experimental part for the HPLC analysis on the columns. This finding simplified considerably the evaluation[5] of a broad biotransformation screening[6] resulting in a selection of optimal biocatalyst and reaction conditions. The CD spectra and specific rotation values of the compounds **1c - 4c** are summarized in Table 5.

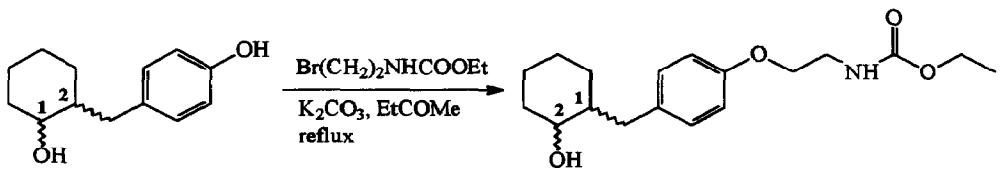
Table 5. CD spectra and specific rotation values of the alcohols **1c - 4c** and **1d - 4d**

Compound	AC	λ (nm)	$\Delta\varepsilon^a$	$[\alpha]_D^{24}$	c(g.100 ml^{-1})^b
1c	1*S*,2*S*	231	-2.7	17.25	0.2
2c	1*R*,2*R*	230	2.83	-18.62	0.46
3c	1*S*,2*R*	223	1.89	35.64	0.2
4c	1*R*,2*S*	221	-2.16	-36.08	0.72
1d	1*S*,2*S*	276.5	-0.08	12.12	0.46
2d	1*R*,2*R*	278	0.06	-11.58	0.2
3d	1*R*,2*S*	280	0.04	16.25	0.63
4d	1*S*,2*R*	280	-0.07	-16.51	0.67

[a] CH_3OH, 0.1 cm, [b] $CHCl_3$

The reason for performing the biotransformation of the MOM protected substrates was: (a) to show that the MTPA esters derived from **1c - 4c** resulting from the biotransformation reactions are stable under the conditions of the HPLC analysis and (b) to improve the chemical yields of the enzyme-mediated hydrolysis. This goal was successfully achieved and, moreover, the substitution of the THP protecting group by the MOM one resulted in a considerable augmentation of the optical purity of the products (Table 1). Chiral precursors **1a - 4a**, prepared and identified in the recent paper (cf. Table 6),[5] were nevertheless suitable intermediates to be used for the following synthesis of the target chiral juvenoids **1d - 4d** as well. The chiral precursors **1a - 4a**

(originated separately from both the preceding preparation[5], and from **1c - 4c**) were alkylated[16] (Scheme 2) using ethyl N-(2-bromoethyl)carbamate[17] in the presence of dry powdered potassium carbonate in refluxing 2-butanone. The chiral juvenoids **1d - 4d** were obtained in chemical yields exceeding 60% (when using acetone as solvent, the yields were much lower, not exceeding 10 %). The absolute configuration of the chiral centers in the juvenoids **1d - 4d** should correspond to those in the starting chiral precursors **1a - 4a.** It is to point out that - according to the IUPAC nomenclature system - the numbering of the saturated ring of the juvenoids **1d - 4d** differs from that of the compounds **1a - 4a** due to the side chain directive carbamate moiety of the compounds **1d - 4d** (Scheme 2). Retention of the absolute configuration was confirmed by evaluation of the ^1H and ^{19}F NMR (Table 3), CD spectra and specific rotation (Table 5) of the juvenoids **1d - 4d** (or the corresponding MTPA esters).



1a (1*S*, 2*S*)
2a (1*R*, 2*R*)
3a (1*S*, 2*R*)
4a (1*R*, 2*S*)

1d (1*S*, 2*S*)
2d (1*R*, 2*R*)
3d (1*R*, 2*S*)
4d (1*S*, 2*R*)

Scheme 2

Table 6. Enantiomeric excess of the starting compounds **1a - 4a** and of the target juvenoids **1d - 4d** determined on basis of the analysis of the corresponding MTPA esters

Starting compound	ee (%)[a]	Product	ee (%)[b]
1a	93	**1d**	95
2a	87	**2d**	90
3a	85	**3d**	90
4a	92	**4d**	95

[a] calculated from the HPLC area,

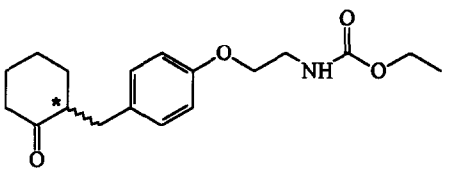
[b] calculated from the integration of the appropriate signals in the ^1H NMR spectra (accuracy ±5%)

Due to a considerable polarity of the MTPA esters derived from the juvenoids **1d - 4d**, the attempt to separate the diastereoisomeric pairs of the MTPA esters using a HPLC was not successful. An alternative method was used to determine the enantiomeric excess of the target chiral juvenoids **1d - 4d**, consisting in evaluation of the integration of the appropriate signals in the ^1H NMR spectra of the corresponding MTPA

esters. However, the alkylation of the chiral phenolic precursors **1a** - **4a** did not influence any of the stereogenic centers and, therefore, it is to expect that the reaction proceeds with retention of the absolute configuration and the enantiomeric excess. This presumption was confirmed by the evaluation of the NMR spectra (Tables 3 and 6).

Table 7. Biological activity values of chiral and racemic juvenoids **1d** - **4d**

Compound	ID_{50} (μg/pupa)
1d	0.000 003 1
1d and 2d (racemic)	0.000 053
2d	0.000 068
3d	0.000 005 2
3d and 4d (racemic)	0.000 12
4d	0.000 72



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The biological testing of chiral juvenoids **1d** - **4d** prepared (cf. Table 7) was performed by a standard method described by Sláma *et al.*[18] The compounds were applied topically on the ventral body part of freshly molted pupae of the yellow mealworm (*Tenebrio molitor*). Biological activity is given in the ID_{50} values (an inhibitory dose) that gives a dose of an active compound causing changes of 50% of observed morphological features at the insect individuals tested. The lower is the ID_{50} value, the better is the biological activity of the juvenoid studied. For comparison, the biological activity values of racemic mixtures of **1d** / **2d** (*cis*-1,2-relative configuration) or those of **3d** / **4d** (*trans*-1,2-relative configuration), prepared earlier[3], were used. Even though the stereoisomeric purity of **1d** - **4d** has been calculated in the range between 85 - 95 % ee, considerable difference in biological activity of the particular stereoisomers **1d** - **4d** has been found. The (1*S*,2*S*)-stereoisomer **1d** generally shows the most favorite activity value when compared with those of other stereoisomers **2d** - **4d** and those of the racemic compounds **1d**/**2d** and **3d**/**4d**. The difference in the biological activity values of the respective stereoisomers **1d** - **4d** shows that the space arrangement of the C(1)- and the C(2)-substituents of the cyclohexane ring plays certain role in the molecular recognition in the interaction of the juvenoid stereoisomers with the receptor active site(s). It is supposed that the receptor decides in favour of

the juvenoid stereoisomers **1d** and **3d** with the (*S*)-absolute configuration of the C(2) stereogenic center. On the other hand, absolute configuration of the C(1) stereogenic center seems to display lower importance in influencing the biological activity (cf. Table 7). This assumption would firmly be supported by comparing the biological activity of the respective enantiomers of ketone **10** or that of its ethylene acetal derivative with each other. It is also favorable that the diastereoisomeric compounds **1d** and **3d** can be produced by a convenient chemoenzymatic process consisting in a *Saccharomyces cerevisiae* mediated reduction of ketone **5c**, followed by an alkylation step.

Experimental

The ^{1}H NMR spectra were recorded on a Varian UNITY-200 spectrometer at 200.06 MHz frequency in deuteriochloroform, using tetramethylsilane as internal reference. The ^{13}C NMR spectra were recorded on a Varian UNITY-500 spectrometer at 125.7 MHz frequency in deuteriochloroform, using central line of the solvent as internal reference (δ = 77.0 ppm). The ^{19}F NMR spectra were recorded on a Varian UNITY-200 spectrometer at 188.15 MHz in deuteriochloroform, with a capillary containing hexafluorobenzene as external reference (δ = 162.9 ppm), unless stated otherwise. The IR spectra were recorded on a Perkin-Elmer 580 instrument in tetrachloromethane, unless stated otherwise. HPLC analyses were carried out on a Knauer instrument. Detection was carried out at 220, 230, 240, 265 nm wavelength using a UV deuterium lamp, and integration was carried out at 220 nm. A column of 250 x 4 (i.d.) mm, filled with Separon SGX (particle size 7 μm) as stationary phase, was used for the analysis. Light petroleum (a 40-68° C boiling fraction) with 3% of ether was used as mobile phase, flow rate 1.4 (*cis*) or 1.2 (*trans* samples) ml.min^{-1}, respectively. Column chromatographies were carried out on silica gel (Herrman, Köln-Ehrenfeld, FRG). Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The CD spectra were obtained from a Jobin Yvon Mark V instrument in methanol. Microanalyses were performed using a Perkin-Elmer 240 C elemental analyser.

2-(4-Methoxymethoxybenzyl)-1-cyclohexanone (5c)

A soln. of 2-(4-hydroxybenzyl)-1-cyclohexanone (0.5 g, 2.4 mmol) in dry benzene (4 ml) was added to a stirred suspension of sodium hydride (0.13 g, 2.7 mmol, a 50% disp. in mineral oil) in dry benzene (5 ml) under nitrogen and the mixture was refluxed for 1 h. The mixture was cooled to 0°C, chloromethyl methyl ether (0.583 g, 7.2 mmol) was added, and the mixture was stirred for 9 h at 0°C. Water (10 ml) was added and the mixture was extracted with diethylether (3 x 100 ml), washed with a 5% aqueous soln. of NaOH (50 ml), then with water (2 x 100 ml), and the organic layer was dried over $MgSO_4$. The volatiles were evaporated *in vacuo*, and the residue (475 mg) was purified by column chromatography on silica gel (50 g) to yield pure **5c** (0.5 g, 82.3%). IR: 3061, 3033, 2996, 2935, 2900, 2863, 2826, 1712, 1612, 1585, 1511, 1449, 1443, 1428, 1278, 1233, 1199, 1176, 1153, 1111, 1081, 1015, 924, 813, 511 cm^{-1} ; ^{1}H NMR: δ 7.07 (m, 2H), 6.94 (m, 2H), 5.14 (s, 2H), 3.47 (s, 3H), 3.16 (dd, J=13.4, 4.1, 1H), 2.38 (dd, 13.4, 9.0, 1H), 2.25-2.60 (m, 2H), 0.90-2.15

(m, 7H); ^{13}C NMR: δ 212.66 (C-1), 52.62 (C-2), 33.38 (C-3), 25.04 (C-4), 28.05 (C-5), 42.16 (C-6), 34.62 (C-7), 133.70 (C-8), 130.06 (C-9, C-13), 116.16 (C-10, C-12), 155.52 (C-11), 94.57 (C-14), 55.93 (C-15); Mass: m/z 248 (M^+, 39), 151 (53), 121 (36), 45 (100); Anal Calcd. for $C_{15}H_{20}O_3$ (248.32): C, 72.55; H, 8.12. Found: C, 72.27; H, 7.98.

cis- and _trans_-2-(4-Methoxymethoxybenzyl)-1-cyclohexanol (8 and 9)

A soln. of **5c** (3.3 g, 13.3 mmol) in dry diethylether (50 ml) was added dropwise to a cooled (0°C) and stirred suspension of lithium aluminum hydride (1.89 g, 49.8 mmol) in dry diethylether (50 ml). After 7 h of stirring, a 25% aqueous soln. of potassium sodium tartrate tetrahydrate (5.4 ml) was added. The mixture was extracted with diethylether (4 x 50 ml), the combined organic extracts were dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude mixture of isomeric alcohols (3.44 g) was separated by column chromatography on silica gel (100 g) to give 0.37 g (11.2%) of pure **8**. IR: 3631, 3061, 3033, 2996, 2897, 2826, 1612, 1585, 1511, 1448, 1232, 1198, 1176, 1153, 1115, 1081, 1018, 1013, 975, 924, 655 cm^{-1} ; ^{1}H NMR: δ 7.10 (m, 2H), 6.95 (m, 2H), 5.15 (s, 2H), 3.79 (dt, 2 x 2.5, 4.0, 1H), 3.48 (s, 3H), 2.66 (dd, J=13.7, 7.6, 1H), 2.49 (dd, 13.7, 7.6, 1H), 1.76 (m, 1H), 1.50-1.70 (m, 3H), 1.35-1.50 (m, 3H), 1.25 (m, 2H); ^{13}C NMR: δ 68.48 (C-1), 43.61 (C-2), 25.30 (C-3), 26.34 (C-4), 20.31 (C-5), 33.26 (C-6), 37.82 (C-7), 134.38 (C-8), 130.02 (C-9, C-13), 116.11 (C-10, C-12), 155.37 (C-11), 94.60 (C-14), 55.93 (C-15); Mass: m/z 250 (M^+, 36), 232 (31), 202 (14), 151 (28), 121 (36), 45 (100); Anal Calcd. for $C_{15}H_{22}O_3$ (250.34): C, 71.97; H, 8.86. Found: C, 72.05; H, 8.98; and 2.40 g (72.3%) of pure **9**. IR: 3624, 3611, 3493, 3061, 3034, 2826, 1612, 1585, 1511, 1448, 1232, 1199, 1176, 1153, 1113, 1080, 1018, 924, 655 cm^{-1} ; ^{1}H NMR: δ 7.10 (m, 2H), 6.95 (m, 2H), 5.15 (s, 2H), 3.48 (s, 3H), 3.28 (td, 2 x 9.6, 4.4, 1H), 3.09 (dd, J=13.4, 4.0, 1H), 2.32 (dd, 13.4, 9.0, 1H), 2.00 (m, 1H), 0.80-1.65 (m, 8H); ^{13}C NMR: δ 74.50 (C-1), 47.08 (C-2), 29.99 (C-3), 25.43 (C-4), 24.90 (C-5), 35.82 (C-6), 38.12 (C-7), 134.08 (C-8), 130.29 (C-9, C-13), 116.07 (C-10, C-12), 155.42 (C-11), 94.64 (C-14), 55.93 (C-15); Mass: m/z 250 (M^+, 30), 232 (24), 202 (10), 151 (16), 121 (25), 45 (100); Anal Calcd. for $C_{15}H_{22}O_3$ (250.34): C, 71.97; H, 8.86. Found: C, 72.11; H, 8.92.

cis- and _trans_-2-(4-Hydroxybenzyl)-1-cyclohexanol (1a - 4a)

A solution of the respective **1c - 4c** (0.1 g, 0.4 mmol) in benzene / ethanol (1 : 1) mixture (5 ml) was heated to 40°C in the presence of conc. hydrochloric acid (0.1 ml) for 12 h. Solvents were evaporated, and the residue was partitioned between water and ether layer. The organic extract was dried over Na_2SO_4, and the residue obtained after removal of the solvent was purified by column chromatography, affording the respective products in 93-96% yields. The following data are common for both **1a** and **2a**: IR: 3445, 3270, 1620, 1520, 1066, 1059, 1042 cm^{-1}; ^{1}H NMR: δ 7.00 (m, 2H), 6.76 (m, 2H), 3.78 (m, w=7.5, 1H), 2.63 (dd, J=7.2, 13.5, 1H), 2.43 (dd, J=7.5, 13.5, 1H), 1.05-1.83 (m, 9H); ^{13}C NMR: δ 68.6 (C-1), 43.6 (C-2), 26.2 (C-3), 25.1 (C-4), 20.3 (C-5), 33.0 (C-6), 37.5 (C-7), 132.2 (C-8), 130.0 (C-9, C-13), 115.0 (C-10, C-12), 154.3 (C-11); Mass:

m/z 206 (M^+, 20), 188 (34), 120 (24), 107 (100); Anal. Calcd. for $C_{13}H_{18}O_2$: C, 75.69; H, 8.79. For **1a** found: C, 75.68; H, 8.71. For **2a** found: C, 75.65; H, 8.74. For **1a**: $[\alpha]_D^{24}$ = + 25.62. For **2a**: $[\alpha]_D^{24}$ = - 24.37. The following data are common for **3a** and **4a**: IR: 3440, 1071, 1041, 1014 cm^{-1}; ^1H NMR: δ 7.05 (m, 2H), 6.74 (m, 2H), 3.29 (dt, J=10.0, 10.0, 4.4, 1H), 3.05 (dd, J=4.0, 13.6, 1H), 2.33 (dd, J=9.2, 13.6, 1H), 1.67-0.80 (m, 9H); ^{13}C NMR: δ 74.58 (C-1), 47.06 (C-2), 30.01 (C-3), 25.43 (C-4), 24.88 (C-5), 35.78 (C-6), 38.10 (C-7), 132.75 (C-8), 130.44 (C-9, C-13), 115.02 (C-10, C-12), 153.65 (C-11); Mass: m/z 206 (M^+, 20), 188 (31), 120 (31), 107 (100); Anal. Calcd. for $C_{13}H_{18}O_2$: C, 75.69; H, 8.79. For **3a** found: C, 75.60; H, 8.72. For **4a** found: C, 75.70; H, 8.77. For **3a**: $[\alpha]_D^{24}$ = + 55.93. For **4a**: $[\alpha]_D^{24}$ = - 57.30. Note: The optical rotation data obtained for **1a - 4a** are in coincidence with those obtained for the same compounds recently.[5]

***cis*- and *trans*-2-(4-Methoxymethoxybenzyl)-1-cyclohexyl acetate (6c and 7c)**

Acetic anhydride (0.272 ml, 2.88 mmol) was added in several portions through a septum to a stirred mixture of **8** (0.7 g, 2.8 mmol) and 4-dimethylaminopyridine (1.2 mg, 0.01 mmol) in dry triethylamine (14 ml) under the room temperature. After 5 h of stirring, the reaction mixture was poured into a cooled saturated soln. of potassium bicarbonate (6 ml). The mixture was extracted by light petroleum (3 x 20 ml), the combined organic extracts were dried over K_2CO_3 and the solvents were evaporated under reduced pressure. The crude product (0.9 g) was purified by column chromatography on silica gel (50 g) affording the pure acetate **6c** (0.732 g, 89.6%). IR: 3062, 3033, 2996, 2897, 2825, 1736, 1612, 1585, 1511, 1449, 1442, 1404, 1311, 1237, 1199, 1176, 1153, 1113, 1080, 1017, 1013, 924, 843, 832 cm^{-1} ; ^1H NMR: δ 7.02 (m, 2H), 6.93 (m, 2H), 5.14 (s, 2H), 4.90 (m, 1H), 3.48 (s, 3H), 2.57 (dd, J=13.7, 6.8, 1H), 2.39 (dd, 13.7, 7.8, 1H), 2.11 (s, 3H), 0.80-2.10 (m, 9H); ^{13}C NMR: δ 72.23 (C-1), 42.34 (C-2), 29.86 (C-3), 20.87 (C-4), 24.94 (C-5), 26.90 (C-6), 37.71 (C-7), 133.84 (C-8), 129.91 (C-9, C-13), 116.14 (C-10, C-12), 155.47 (C-11), 94.57 (C-14), 55.93 (C-15), 170.80 and 21.31 (OAc); Mass: m/z 292 (M^+, 27), 232 (65), 202 (10), 151 (13), 121 (19), 107 (20), 45 (100); Anal Calcd. for $C_{17}H_{24}O_4$ (292.38): C, 69.84; H, 8.27. Found: C, 69.12; H, 8.38. The same procedure was used for the preparation of **7c** (0.599 g, 78.6% yield) starting from the corresponding alcohol **9** (0.652 g, 2.6 mmol). IR: 3061, 3034, 2826, 1735, 1613, 1585, 1511, 1450, 1405, 1312, 1241, 1199, 1176, 1154, 1119, 1081, 1018, 1013, 924, 838 cm^{-1} ; ^1H NMR: δ 7.04 (m, 2H), 6.94 (m, 2H), 5.15 (s, 2H), 4.58 (dt, J=10.0, 4.5, 1H), 3.48 (s, 3H), 2.84 (dd, J=13.5, 4.0, 1H), 2.28 (dd, 13.5, 9.0, 1H), 2.02 (s, 3H), 1.98 (m, 1H), 0.80-1.80 (m, 8H); ^{13}C NMR: δ 76.90 (C-1), 43.77 (C-2), 30.00 (C-3), 25.07 (C-4), 24.51 (C-5), 31.84 (C-6), 38.03 (C-7), 133.66 (C-8), 130.05 (C-9, C-13), 116.05 (C-10, C-12), 155.39 (C-11), 94.57 (C-14), 55.91 (C-15), 170.86 and 21.27 (OAc); Mass: m/z 292 (M^+, 17), 232 (49), 202 (11), 151 (11), 121 (17), 107 (19), 45 (100); Anal Calcd. for $C_{17}H_{24}O_4$ (292.38): C, 69.84; H, 8.27. Found: C, 69.32; H, 8.43.

***cis*-(1*S*,2*S*)-, *cis*-(1*R*,2*R*)-, *trans*-(1*S*,2*R*)- and *trans*-(1*R*,2*S*)-2-(4-Methoxymethoxybenzyl)-1-cyclohexanols (1c - 4c)**

The chiral alcohols **1c - 4c** were obtained by a biotransformation of the respective substrates **5c - 7c**. The biotransformation reactions are described in Table 2 in more details. The CD spectra and the specificic rotation values of **1c - 4c** are summarized in Table 5. Chemical yields of the biotransformation reactions are summarized in Table 1. The IR, ^{1}H NMR, ^{13}C NMR, mass spectra and microanalyses of stereoisomers **1c - 4c** were found to be in good accordance with the data found for the racemic alcohols **8** and **9**.

MTPA esters of alcohols 1c - 4c and 1d - 4d

A general procedure used for the preparation of the MTPA esters in a milligram scale starting from the chloride of MTPA has already been described in details.[19] Characterization of the MTPA esters by the spectral data is summarized in Table 3. The HPLC-based determination of the optical purity of the alcohols **1c - 4c** using their diastereoisomeric MTPA esters is presented in Table 4. Enantiomeric excess of the chiral juvenoids **1d - 4d** was determined on the basis of integration of selected signals in the ^{1}H NMR spectra of the corresponding MTPA esters (Table 6).

(1*S*,2*S*)-*cis*-, (1*R*,2*R*)-*cis*-, (1*R*,2*S*)-*trans*- and (1*S*,2*R*)-*trans*-Ethyl N-{2-[4-(2-hydroxy-1-cyclohexylmethyl)-phenoxy]ethyl}carbamates (1d - 4d)

Dry powdered potassium carbonate (1 g) and ethyl N-(2-bromoethyl)carbamate (1g, 5.0 mmol) were added to a soln. of the respective chiral precursor **1a - 4a** (0.2 mmol) in 2-butanone (15 ml), the mixture was refluxed for 16 h, then cooled and filtered. The solid was washed with diethylether (30 ml), and then the filtrate was washed with water (10 ml) and dried over $MgSO_4$. The volatiles were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel, yielding the respective compounds **1d** (62.5%), **2d** (63.6%), **3d** (97.6%) or **4d** (98.0%). The CD spectra and the specific rotation values of **1d - 4d** are summarized in Table 5. The following data are common for both **1d** and **2d**. IR ($CHCl_3$): 3616, 3454, 1714, 1611, 1584, 1519 sh, 1510, 1447, 1240, 1177, 1014, 973 cm^{-1}; ^{1}H NMR: δ 7.10 (m, 2H), 6.80 (m, 2H), 5.20 (bs, 1H), 4.12 (q, J=7.1, 2H), 4.00 (t, J=5.1, 2H), 3.78 (dt, J=2 x 2.6, 4.4, 1H), 3.56 (bq, J=5.2, 2H), 2.65 (dd, J=13.6, 7.5, 1H), 2.48 (dd, J=13.6, 7.8, 1H), 1.26-1.83 (m, 9H), 1.24 (t, J=7.1, 3H); ^{13}C NMR: δ 68.28 (C-1), 43.51 (C-2), 26.10 (C-3), 25.07 (C-4), 20.21 (C-5), 33.00 (C-6), 37.46 (C-7), 133.51 (C-8), 129.80 (C-9, C-13), 114.05 (C-10, C-12), 156.63 (C-11), 66.73 (C-14), 40.35 (C-15), 156.47 (C-16), 60.70 (C-17), 14.32 (C-18); MS: m/z 321 (M^+, 6), 116 (100), 88 (32), 44 (6); Anal. Calcd. for $C_{18}H_{27}O_4N$ (321.41): C, 67.26; H, 8.47; N, 4.36. Found for **1d**: C, 67.03; H, 8.39; N, 4.39; and for **2d**: C, 67.37; H, 8.42; N, 4.45. The following data are common for both **3d** and **4d**. IR ($CHCl_3$): 3619, 3453, 3385 sh, 1709, 1611, 1584, 1519 sh, 1510, 1448, 1239, 1030 cm^{-1}; ^{1}H NMR: δ 7.08 (m, 2H), 6.80 (m, 2H), 5.24 (bs, 1H), 4.11 (q, J=7.1, 2H), 4.00 (t, J=5.1, 2H), 3.56 (bq, J=5.2, 2H), 3.27 (dt, J=2 x 10.0, 4.4, 1H), 3.08 (dd, J=13.6, 3.9, 1H), 2.31 (dd, J=13.5,

9.0, 1H), 1.28-1.61 (m, 9H), 1.24 (t, J=7.1, 3H); ^{13}C NMR: δ 74.36 (C-1), 47.00 (C-2), 29.86 (C-3), 25.36 (C-4), 24.84 (C-5), 35.76 (C-6), 37.96 (C-7), 133.20 (C-8), 130.26 (C-9, C-13), 114.15 (C-10, C-12), 156.62 (C-11), 66.93 (C-14), 40.65 (C-15), 156.56 (C-16), 60.92 (C-17), 14.62 (C-18); MS: m/z 321 (M^+, 6), 116 (100), 88 (24); Anal. Calcd. for $C_{18}H_{27}O_4N$ (321.41): C, 67.26; H, 8.47; N, 4.36. Found for **3d**: C, 67.19; H, 8.29; N, 4.41; and for **4d**: C, 67.32; H, 8.59; N, 4.42.

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References

1. Henrick, C. A.; Anderson, R. J.; Staal, G. B.; Ludvik, G. F. *J. Agric. Food Chem.* **1978**, *26*, 542.
2. Wimmer, Z.; Romaňuk, M. *Collect. Czech. Chem. Commum.* **1989**, *54*, 2302.
3. Wimmer, Z.; Streinz, L.; Romaňuk, M. *Collect. Czech. Chem. Commum.* **1985**, *50*, 2453; Wimmer, Z.; Šaman, D.; Němec, V.; Francke, W. *Helv. Chim. Acta*, **1994**, *77*, 561.
4. e. g. Wimmer, Z.; Romaňuk, M.; Kuldová, J.; Hrdý, I.; Sehnal, F. in *Insect Chemical Ecology* (Hrdý, I., Ed.), SPB Acad. Publ. - Academia, **1991**, p. 453.
5. Zarevúcka, M.; Rejzek, M.; Wimmer, Z.; Šaman, D.; Streinz, L. *Tetrahedron* **1993**, *49*, 5305.
6. Zarevúcka, M.; Rejzek, M.; Wimmer, Z. to be published.
7. Pinnick, H. W.; Lajis, N. H. *J. Org. Chem.* **1978**, *43*, 3964.
8. Wimmer, Z.; Romaňuk, M. *Collect. Czech. Chem. Commun.* **1981**, *46*, 2573.
9. Kluge, A. F.; Untch, K. G.; Fried, J. H. *J. Amer. Chem. Soc.* **1972**, *94*, 7827.
10. Wimmer, Z.; Buděšínský, M.; Macek, T.;Svatoš, A.; Šaman, D.; Vašíčková, S.; Romaňuk, M. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2326.
11. Rinaldi, P. L. *Prog. Nucl. Magn. Reson. Spectrosc.* **1982**, *15*, 291.
12. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
13. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, 95, 512.
14. Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143.
15. Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1970**, *35*, 4002.
16. Rejzek, M. *Dissertation Thesis*, IOCB Prague, **1994**.
17. Rejzek, M.; Zarevúcka, M.; Wimmer, Z. *BioMed. Chem. Lett.* **1992**, *2*, 963.
18. Sláma, K.; Romaňuk, M.; Šorm, F. in *Insect Hormones and Bioanalogues*, Springer Vienna, **1974**.
19. Minamikawa, J.; Brossi, A. *Tetrahedron Lett.* **1978**, 3085.

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