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Diastereo- and enantioselective bioreduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate clofibrate analogues by *Kluyveromyces marxianus* and other whole cell biocatalysts

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Abstract—Growing and resting cells of several yeasts, which catalyze the hydride transfer to a carbonyl, were screened and used in conditions to find out the suitable methodology to prepare clofibrate analogues. Clofibrate is an antilipidemic drug. In particular, the bioreduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate **1** was investigated to separately prepare the four possible stereoisomers of the ethyl 2-(4-chlorophenoxy)-3-hydroxybutanoate **2**. Compound (2*R*,3*S*)-**2** was prepared with ee = 97% and 73% yield in the presence of *Kluyveromyces marxianus*; (2*S*,3*S*)-**2** preparation with ee >99% in 9% and 33% yield was mediated by *Saccharomyces cerevisiae* CBS 7336 and *Trigonopsis variabilis*, respectively. Diastereomeric excess values of all the reactions investigated were up to >99%. Furthermore, enantiomeric excesses of the bioconversions varied between 2% and >99% using growing cells and, 12% and >99% using resting cells. The absolute configuration of (2*R*,3*S*)-**2** was established by X-ray analysis of the corresponding acid **3**.

1. Introduction

Chiral alcohols are very important intermediates in organic synthesis and precursors of a large number of pharmaceuticals.[1–5] One of the easiest and widely used synthetic routes to prepare this crucial class of compounds is the chemical and chemo-enzymatic asymmetric reduction of prochiral ketones. Various chiral reducing agents[6–9] or chirally modified boron- and aluminium-hydrides for hydride transfer,[10,11] catalytic reduction with chiral transition metal complexes[12–18] are the most used chemical methods to enantioselectively reduce the carbonyl. Chemo-enzymatic hydride transfer represents a valuable alternative to chemical methodologies.[19,20] The success of such biotransformations is mainly due to the presence in the cells of enzymes, involved in the reaction, capable of mediating such a hydride transfer with very high regio- and stereoselectivity.[21] Furthermore, biochemical approaches usually consist of mild reaction conditions and make use of environmentally safe reagents.[22] An increasing number of microorganisms have shown the ability of mediating the reduction reaction of the carbonyl with high stereoselectivity.[23]

Clofibrate [ethyl 2-(4-chlorophenoxy)-2-methylpropanoate], a well-known lipid-modifying agent, has been proven to be active in the treatment of human dyslipaemias[24–28] and more recently in rat pressure overload induced cardiac hypertrophy.[29] Nevertheless, it shows, especially in humans, a number of adverse side effects.[30–33] Hence, the development of new clofibrate analogues with an improved pharmacological profile,[34–36] to efficaciously cure the hyperlipidaemias and other cardiovascular diseases is crucial. A new series of chiral clofibrate analogues has been prepared by us as racemates and has already been tested. Unfortunately, they were inactive in a transactivation assay aimed at

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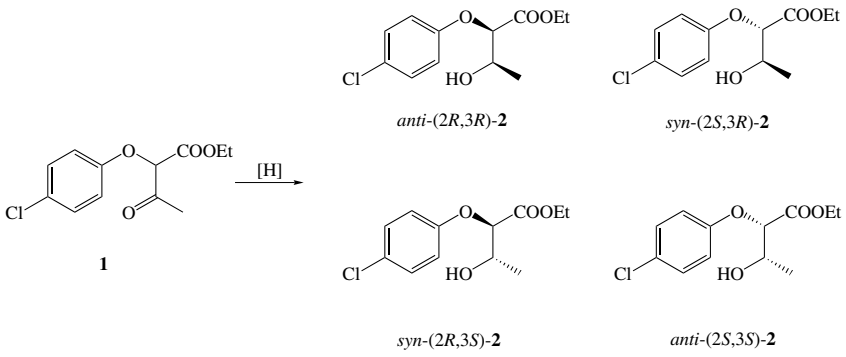


Figure 1. The stereoisomers of ethyl 2-(4-chlorophenoxy)-3-hydroxybutanoate **2** obtainable by the reduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate **1**.

evaluating their capacity to interact with peroxisome proliferator-activated receptor isoform α (PPARα).[37] Thus, single stereoisomers were needed for the bioassay, to check the hypothesis that the lack of activity was not due to molecular structural features, but due to a crowding in the ligand binding domain of the receptor, as the racemate of each compound was assayed.

The main challenge in the preparation of **2**, in an enantiomerically pure form, is represented by the presence of two stereogenic centres in the molecule, involving the possible formation of four stereoisomers (Fig. 1).

Growing and resting cells of different yeast strains have been used, as a tool for the diastereo- and enantioselective reduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate **1** and with the purpose of finding a procedure to possibly obtain separately the four stereoisomers of **2** in high chemical yield and high enantiomeric excess.

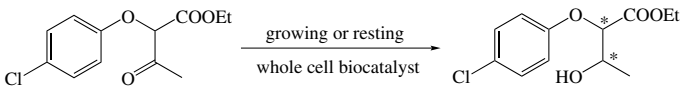
2. Results and discussion

Baker's yeast is commonly employed in bioreduction reactions because of its simplicity of use. Thus, as a first attempt, commercially available Baker's yeast was used by us.[38] In this case, we observed a quantitative conversion of the substrate ($c = 5$ g/L) into the product with very high diastereomeric and enantiomeric excess (de = 92%, $ee_{syn\ pair}$ = 94%). However, in principle looking for better results and eventually reverse stereoselectivity, many other microorganisms may be used to induce the same transformation. Next, different yeast strains were screened (Scheme 1) to improve the process mediated by Baker's yeast, and to possibly obtain separately the four stereoisomers of **2**.

Kluyveromyces marxianus CBS 6556, *Saccharomyces cerevisiae* DSM 11285, *Saccharomyces cerevisiae* CBS 7336, *Cryptococcus curvatus* ATCC 29509, *Candida bombicola* ATCC 22214, *Trigonopsis variabilis* DSM 70714 were then selected for scaling up the reduction of **1**. The reactions were performed by using both growing and resting cells. The results obtained are reported in Tables 1 and 2, respectively.

With growing cells, all of the strains showed moderate to high diastereo- and enantioselectivity (Table 1). Notable conversions were obtained for *T. variabilis* (80%), *S. cerevisiae* DSM (89%), *C. bombicola* (91%) and for *K. marxianus* (quantitative), whereas it was lower (59%) for *S. cerevisiae* CBS. When *Cr. curvatus* was employed, the extent of conversion was only 14%.

In all cases, the main pair of stereoisomers turned out to be the *syn* form, with diastereomeric excesses ranging from 32% (*T. variabilis* and *Cr. curvatus*) to >99% (*K. marxianus*). The absolute configuration of **2** formed in the bioreduction performed in the presence of *K. marxianus* was established to be (2*R*,3*S*) by single crystal X-ray analysis of the corresponding acid **3** (quantitatively obtained by treating **2** with KOH) (Fig. 2). All the other absolute configurations were assessed by comparing the HPLC retention times of standard compounds prepared from threonines.[39] (2*R*,3*S*)-**2** was the most abundant enantiomer within the *syn* pair formed in the presence of all the microorganisms listed in Table 1. Ee's varied from 42% with *T. variabilis* to 92% with *S. cerevisiae* DSM 11285. In the case of *S. cerevisiae* CBS 7336, almost racemic *syn*-**2** (ee = 2%) was obtained. Within the *anti* pair, (2*S*,3*S*)-**2** was the only enantiomer formed in the reduction mediated by *S. cerevisiae* CBS 7336 and *T. variabilis* (>99%), even if in low yields (9% and 33%, respectively). Ee = 42% for *C. bombicola*.



Scheme 1. Bioreduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate **1**.

Table 1. Reduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate **1** with growing cells of different microorganisms

Microorganism	t_R (h)	Yield[a] (%)	Conv.[b] (%)	De[c] (%)	Ee_{syn} [d] (%)	Ee_{anti} [d] (%)	$[\alpha]_D^{20}$
S. cerevisiae DSM	8	61	89	81	92	66	+27.6
S. cerevisiae CBS	24	40	59	83	2	>99	+18.8
C. bombicola	24	53	91	56	72	42	+24.3
T. variabilis	29	79	80	32	42	>99	+24.4
Cr. curvatus	25	5	14	32	ND[e]	ND[e]	+0.8
K. marxianus	8	73	Quantitative	>99	86	—	+31.3

[a] Yields refer to the product isolated by chromatography.
[b] Conversion = percentage of conversion (^{1}H NMR data).
[c] Diastereomeric excesses (*syn* > *anti* pair) determined by ^{1}H NMR.
[d] Enantiomeric excesses were determined by HPLC.[39]
[e] ND = not determined due to low conversion.

Table 2. Reduction of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate **1** with resting cells of different microorganisms

Microorganism	t_R (h)	Yield[a] (%)	Conv.[b] (%)	De[c] (%)	Ee_{syn} [d] (%)	Ee_{anti} [d] (%)	$[\alpha]_D^{20}$
S. cerevisiae DSM	3.5	79	Quant.	84	85	24	+51.0
S. cerevisiae CBS	2	41	95	87	63	>99	+13.3
C. bombicola	5	53	95	54	93	66	+12.5
T. variabilis	2.5	86	94	27	98	55	+13.3
Cr. curvatus	4	31	43	11	98	12	+5.6
K. marxianus	1.5	84	Quant.	>99	97	—	+48.9

[a] Yields refer to the product isolated by chromatography.
[b] Conversion = percentage of conversion (^{1}H NMR data).
[c] Diastereomeric excesses (*syn* > *anti* form) determined by ^{1}H NMR.
[d] Enantiomeric excesses were determined by HPLC.[39]

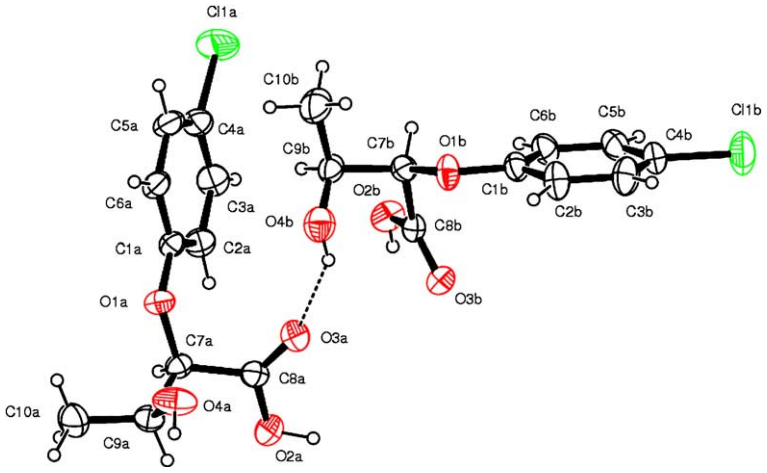


Figure 2. ORTEP view of the asymmetric unit with the atomic numbering scheme of (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoic acid **3**. Thermal ellipsoids probability level at 30%.

It is noteworthy that the enantioselectivity and enzymatic activity (different reaction times) depended on the strain involved in the biotransformation, while the use of a different strain of the same microorganism, as in the case of *S. cerevisiae*, led to quite different results in terms of conversion (89% and 59%, respectively). These differences can be ascribed to the variable extent of expression of the enzymes involved in the bioreduction.[21]

The best results were obtained when using resting cells of *K. marxianus*, which have proven to be useful in bioreductions.[40–42] In this case, a complete diastereoselectivity and a high enantioselectivity was observed, the (2*R*,3*S*) being the major stereoisomer formed. This result is interesting if compared to a recently reported reduction of β-keto ester precursor of β-lactam group antibiotics, in which a (3*R*)-3-hydroxy compound was formed by reducing the carbonyl group in the presence of *K. marxianus* KCTC 7155.[43] Furthermore, by considering the reaction times, *K. marxianus* shows the highest reducing activity together with *S. cerevisiae* DSM 11285.

Biotransformations performed using resting cells were faster than the corresponding reactions accomplished with growing cells (Table 2). Diastereoselectivity was

almost similar under the two conditions, with the exception of *Cr. curvatus*, for which a lower de value (11% with resting cells and 34% with growing cells) was obtained. Enantioselectivity improved; in many cases the ee values were higher than those reported in Table 1. A significant increase in the enantioselectivity was again obtained with *K. marxianus*, by which it was possible the preparation of almost one out of the four stereoisomers: ethyl (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoate. The use of *S. cerevisiae* CBS 7336 caused a remarkable increase of ee_{syn} = 63% when compared to 2% obtained using growing cells. When using the resting cells of *T. variabilis*, (2*R*,3*S*)-**2** was almost the only stereoisomer formed within the *syn* pair (ee = 98%), while a much lower enantiomeric excess (ee = 42%) was obtained using growing cells.

3. Conclusion

Growing and resting cells of several yeasts were screened to find out the best biosystem to diastereo- and enantioselectively reduce the ethyl 2-(4-chlorophenoxy)-3-oxobutanoate **1** into ethyl 2-(4-chlorophenoxy)-3-hydroxybutanoate **2**.

Kluyveromyces marxianus was found to be the best microorganism for accomplishing such a transformation, as it nearly afforded enantiomerically pure (2*R*,3*S*)-**2**. Its absolute configuration was unambiguously established by single crystal X-ray analysis of the corresponding acid **3**, quantitatively obtained by hydrolysis of (2*R*,3*S*)-**2** isolated from the reduction reaction performed in the presence of *K. marxianus*.

Work is currently in progress aimed at producing the four enantiomerically pure stereoisomers of **2** by screening a higher number of known and not yet reported microorganisms with reducing activity. Furthermore, attempts to isolate the enzyme responsible of *K. marxianus* reductase activity and having a reverse stereochemical preference with respect to the already reported isolated enzyme[44] are also underway.

4. Experimental

4.1. General

Melting points were taken on electrothermal apparatus and are uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian Mercury 300 MHz spectrometer with chemical shifts reported in parts per million (δ). Absolute values of the coupling constant (J) are reported. IR spectra were also recorded on a Perkin–Elmer 681 spectrometer. Reaction progress was monitored by TLC or GC analyses. Thin-layer chromatography (TLC) was performed on silica gel sheets with a fluorescent indicator (Statocrom SIF, 60 F_{254} Merk); TLC spots were observed under ultraviolet light or visualized with I_2 vapour. Column chromatography was conducted using silica gel Merk 60 (0.063–0.200 μm). GC analyses were performed by using a HP-5MS column (5% phenyl methyl siloxane; 30 m × 0.321 mm × 0.25 μm) on an Agilent 6850 Series GC System. GC–MS analyses were performed on a Hewlett–Packard 6890-5793MSD, and microanalysis on an Elemental Analyzer 1106–Carlo Erba-instrument. Optical rotations were determined on a Perkin–Elmer model 341 polarimeter (c = 1 g/100 mL, $CHCl_3$).

The ee's and absolute configurations of the reaction products were determined by HPLC analysis performed on a Perkin–Elmer 200 series with a UV/vis detector 785A on a commercially available Chiralcel OD (Daicel) in isocratic conditions employing *n*-hexane–2-propanol = 98:2, flow rate 0.8 mL/min, λ = 230 nm.

The absolute configuration of (2*R*,3*S*)-**3** was established by single crystal X-ray analysis (see below). Absolute configurations of **2** were assigned by HPLC under the conditions used to determine the ee values.[39]

4.2. Chemicals

Compound **1** was prepared by reacting ethyl 2-chloro-3-oxobutanoate (Aldrich Chemical Co.) and caesium 4-chlorophenate. Caesium 4-chlorophenate was prepared by adding to a solution of 4-chlorophenol (10.51 g, 8.17 mmol) in absolute ethanol (15 mL) a suspension of Cs_2CO_3 (8.17 mmol) in absolute EtOH (35 mL). The mixture was stirred for 3 h at room temperature. EtOH was then removed under reduced pressure and a white powder was obtained.

4.2.1. Preparation of ethyl 2-(4-chlorophenoxy)-3-oxobutanoate 1. Ethyl 2-chloro-3-oxobutanoate (18.22 mmol) and caesium 4-chlorophenate (25.52 mmol), were stirred at 50 °C. The reaction was stopped after 4 h (GC analysis). The reaction mixture was treated with ethyl acetate and then washed with saturated aq NaCl. The organic extract was dried over anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure. The product was isolated by chromatography (silica gel; mobile phase: petroleum ether/ethyl acetate = 9:1); 69% yield; keto form/enol form = 68:32 by ^{1}H NMR. Oil. IR (neat): 3600–3150, 3100, 3073, 2985, 2940, 2875, 1758, 1735, 1661, 1629, 1587, 1489, 1446, 1414, 1371, 1268, 1216, 1167, 1089, 1013, 827, 665 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 11.32 (s, 1H, OH enol: exchanges with D_2O); 7.26–7.20 (m, 4H, aromatic protons, 2H of keto form and 2H of enol form), 6.87–6.81 (m, 4H, aromatic protons, 2H of keto form and 2H of enol form), 5.03 (s, 1H, *CH*), 4.30–4.23 (q, J = 7.14 Hz, 2H, CH_2CH_3, keto form), 4.22–4.15 (q, J = 7.14 Hz, 2H, CH_2CH_3, enol form), 2.35 (s, 3H, $COCH_3$, keto form); 1.95 (s, 3H, $COCH_3$, enol form), 1.29–1.24 (t, J = 7.14 Hz, 3H, CH_3CH_2, keto form); 1.17–1.13 (t, J = 7.14 Hz, 3H, CH_3CH_2, enol form). ^{13}C NMR ($CDCl_3$): δ 200.66, 165.88, 155.44, 129.95, 129.63, 127.87, 116.71, 116.05, 83.16, 62.79, 26.56, 14.22. GC–MS (70 eV) (*m*/*z*) (rel. int.) 258 [M(^{37}Cl)$^+$, 10], 256 [M(^{35}Cl)$^+$, 29], 216 (10), 214 (30), 147 (15), 143 (32), 141 (100), 139 (29), 129 (8), 128 (9), 113 (11), 111 (22), 75 (15), 43 (37). Anal. Calcd for $C_{12}H_{13}ClO_4$: C, 56.25; H, 5.08. Found: C, 56.21; H, 5.06.

4.3. Microorganism sources

K. marxianus CBS 6556, *S. cerevisiae* DSM 11285, *S. cerevisiae* CBS 7336, *Cr. curvatus* ATCC 29509, *C. bombicola* ATCC 22214 and *T. variabilis* DSM 70714 were obtained from public type culture collections (ATCC, CBS, DSM).

4.4. Culture medium

The microorganisms were cultivated under aerobic conditions in a medium containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1% glucose. To the same medium, 2% Agar-Agar was added for cells preservation on agar slants.

4.5. Bioreduction methodologies

4.5.1. Baker's yeast-induced bioreduction. Baker's yeast (2.5 g) was dispersed to give a smooth paste in tap water (4.5 mL). Ethyl 2-(4-chlorophenoxy)-3-oxobutanoate **1** (25 mg) dissolved in ethanol (0.5 mL) was added and stirred at 30 °C in a LAB-LINE orbital shaker (250 rpm). The initial pH was 4.16. The reaction progress was monitored by GC. After three hours, the reaction was stopped by centrifugation, decantation and extraction by EtOAc. The extracts were dried over anhydrous Na_2SO_4 and the solvent evaporated under reduced pressure. A yellow oil was obtained.

4.5.2. Bioreduction using growing cells. Cells preserved on agar slants at 4 °C were used to inoculate a 250 mL flask containing the cultivation medium (100 mL). The flask was incubated aerobically at 30 °C on an orbital shaker (250 rpm). The 250 mL flask containing the cultivation medium (100 mL) was then inoculated with the 24 h old suspension (5 mL) and incubated under the same conditions for 24 h. A 1 L flask containing the same cultivation medium (400 mL) was then inoculated with the latter suspension (5 mL) and incubated for 24 h. Ethyl 2-(4-chlorophenoxy)-3-oxobutanoate **1** (200 mg) dissolved in ethanol (1 mL) was then added to the culture. Reaction progress was monitored by GC analysis and stopped at the times indicated in Table 1. The content of the flask was then centrifuged and the supernatant extracted with ethyl acetate. All the reactions were repeated at least twice without any noticeable bias in the results.

4.5.3. Bioreduction using resting cells. Culture conditions were the same as in the case of the growing cells. Once cell growth reached the stationary phase, cells were harvested by centrifugation (20 min and 4000 rpm) at 4 °C. The cells were then recovered and washed three times (20 mL each time) with 0.1 M KH_2PO_4 buffer pH = 7. The cells were sedimented by centrifugation as previously described. The cell wet mass, free from culture medium, was re-suspended in the same buffer solution enriched with 1% glucose to reach a concentration of 0.5 g/L. Then, substrate **1** (2 mM final concentration) was added to the reaction medium. The flask was shaken (250 rpm) in an orbital shaker and kept at 30 °C. The reaction progress was monitored by GC analysis and stopped at times indicated in Table 2. The contents of the flask were then centrifuged and the supernatant extracted with ethyl acetate. All the reactions were repeated at least twice without any noticeable change in the results.

4.5.4. Ethyl 2-(4-chlorophenoxy)-3-hydroxybutanoate 2. Oil. IR (neat): 3500–3100, 3061, 2982, 2933, 1738, 1596, 1584, 1492, 1375, 1283, 1238, 1200, 1094, 1023, 825 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.26–7.18 (m, 4H, aromatic protons, 2H for each stereoisomer pair), 6.88–6.78 (m, 4H, aromatic protons, 2H for each stereoisomer pair), 4.56–4.53 (d, J = 4.29 Hz, 1H, C*H*OC_6H_4Cl of the *anti* pair), 4.43–4.41 (d, J = 4.94 Hz, 1H, C*H*OC_6H_4Cl of the *syn* pair) 4.30–4.17 (m, 6H, 3H for each pair of stereoisomers: 1H of C*H*CH_3 and 2H of CH_2CH_3), 2.90–2.50 (br s, 2H, 1H for each pair of stereoisomers, OH: exchange with D_2O), 1.34–1.32 (d, J = 6.45 Hz, 3H, CHC*H*$_3$ of the *anti* pair), 1.33–1.31 (d, J = 6.45 Hz, 3H, CHC*H*$_3$ of the *syn* pair), 1.25–1.21 (t, J = 7.14 Hz, 3H, C*H*$_3$CH$_2$ of the *anti* pair), 1.24–1.20 (t, J = 7.14 Hz, 3H, C*H*$_3$CH$_2$ of the *syn* pair). ^{13}C NMR ($CDCl_3$): δ 169.76, 156.45, 129.76, 127.29, 116.88, 81.52, 81.06, 68.65, 68.60, 61.95, 19.10, 14.37. GC–MS (70 eV) (*m*/*z*) (rel. int.) 260 [M(^{37}Cl)$^+$, 5], 258 [M(^{35}Cl)$^+$, 17], 214 (21), 143 (30), 141 (100), 139 (16), 130 (17), 128 (52), 111 (13), 75 (12), 43 (13). Anal. Calcd for $C_{12}H_{15}ClO_4$: C, 55.81; H, 5.81. Found: C, 55.84; H, 5.83.

4.6. Preparation of (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoic acid 3

To (2*R*,3*S*)-**2** (1.357 mmol) in THF (18 mL), a solution of KOH (152 mg, 2.713 mmol, in 6 mL water) was added. The resulting mixture was stirred at room temperature for 1 h. Reaction progress was monitored by GC analysis. THF was then removed under reduced pressure and the alkaline solution washed three times with ethyl ether, acidified with 2 M HCl and extracted three times with ethyl ether. The second extract was combined and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and a colourless oil obtained. The crude product was purified by crystallization (chloroform/hexane). Quantitative yield. Mp: 105–106 °C ($CHCl_3$/hexane). $[\alpha]_D^{20} = +12.4$ (c 1.0, $CHCl_3$). IR (KBr): 3700–3200, 3014, 2981, 2926, 2855, 1706, 1593, 1584, 1493, 1374, 1286, 1241, 1175, 1132, 1110, 1089, 1075, 1018, 886, 829 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 7.22–7.21 (m, 2H, aromatic protons), 7.20–7.00 (br s, 2H, COO*H* and O*H*: exchange with D_2O), 6.82–6.80 (m, 2H, aromatic protons), 4.48–4.47 (d, J = 3.58 Hz, 1H, C*H*OC_6H_4Cl), 4.36–4.29 (qd, J = 6.33 Hz and 3.58 Hz, 1H, C*H*OH), 1.37–1.36 (d, J = 6.33 Hz, 3H, C*H*$_3$CHOH). ^{13}C NMR (76 MHz, $CDCl_3$): δ 173.38, 156.04, 129.61, 127.33, 116.57, 80.33, 68.54, 19.09. Anal. Calcd for $C_{10}H_{11}ClO_4$: C, 52.07; H, 4.81. Found: C, 52.06; H, 4.85.

4.7. X-ray analysis

To establish the absolute configuration at C(7) and C(9) in an unambiguous manner, suitable crystals were

grown and subjected to single crystal X-ray analysis, using a Nonius Kappa CCD area detector diffractometer equipped with a fine focus sealed graphite-monochromated Mo-K_α radiation ($\lambda = 0.71073$ Å). Data for (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoic acid **3** were collected at 293(2) K. Data reduction and cell refinement were carried out with the programs DENZO[45] and COLLECT.[46] The structure was solved by the direct methods procedure of SIR97,[47] while the refinement processes were carried on full matrix least squares technique using SHELXL-97.[48] Detailed crystal data and geometrical parameters have been deposited in the supporting information (cif file).[49] The asymmetric unit of (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoic acid **3** with the atomic numbering scheme is depicted in Figure 2.

Pertinent crystallographic data for (2*R*,3*S*)-2-(4-chlorophenoxy)-3-hydroxybutanoic acid **3**: $C_{10}H_{11}ClO_4$, M_r = 230.64 g cm^{-3}, orthorhombic, space group: $P2_12_12$, a = 13.2109(4), b = 24.8989(9), c = 6.8380(2) Å, cell volume = 2244.75(12) Å^3, $Z = 8$, $T = 293(2)$ K, ρ_c = 1.365 g cm^{-3}, $\mu = 0.331$ mm^{-1}, θ range = 2.25°–27.52°, *hkl* indices $-17 \leqslant h \leqslant 17$, $-31 \leqslant k \leqslant 32$, $-8 \leqslant l \leqslant 8$, reflections (measured) = 12,009, reflections (unique) = 5031, reflections (unique [$F_o > 2\sigma\{|F_o|\}$]): 2563, $R_{int} = 0.052$, 344 parameters, R_1/wR_2 (all data): 0.1433/0.1059, R_1/wR_2 ($I > 2\sigma(I)$): 0.0529/0.0824, Flack parameter = −0.02(8), largest diff. peak/hole: 0.162/−0.245 e Å^{-3}.

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