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Enantioselective biotransformations of racemic 2-aryl-3-methylbutyronitriles using *Rhodococcus* sp. AJ270

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Abstract

Rhodococcus sp. AJ270 is a useful biocatalyst for the synthesis of some enantiopure *S*-(+)-2-aryl-3-methylbutyric acids and *R*-(+)-2-aryl-3-methylbutyramides from the hydrolysis of 2-aryl-3-methylbutyronitriles under mild conditions. The nitrile-hydrolyzing enzymes involved in this novel microorganism are very sensitive to the steric effect of the *para*-substituent on the aromatic ring. While the nitrile hydratase displays a low *S*-enantioselectivity against nitriles, the amidase has a strict *S*-enantioselectivity against 2-aryl-3-methylbutyramides.

Keywords: Enantioselective biotransformations; 2-Aryl-3-methylbutyronitriles; *S*-(+)-2-aryl-3-methylbutyric acids; *R*-(–)-2-aryl-3-methylbutyramides

1. Introduction

There is a renaissance of interest in the biotransformations using microorganisms and isolated enzymes in organic synthesis [1–5]. For a recent comprehensive treatise, see [5]. Since most of the reactions are highly chemo-, regio-, and stereoselective, biotransformations offer many opportunities for the syntheses of compounds which are sometimes not readily obtainable by 'conventional' chemical means [2–5]. Furthermore, almost all biotransformations are performed under very mild conditions and biocatalysts are biodegradable, biotransformations are regarded as environmentally benign processes [6–8]. Moreover, with the rapid development of biological science and technology, biocatalysis can now be engineered to furnish high efficiency and improved selectivities [1,9].

Nitriles are important intermediates in organic synthesis because of their easy availability and versatile transformations [10]. Chemical hydrolyses of nitriles, for instance, are used in both laboratory and industry to prepare amides and acids. Unfortunately, these applications are restricted to structurally simple nitriles bearing no labile groups, because chemical processes often require harsh conditions such as using strong acids or bases, and give poor selectivities. In contrast, biotransformations of nitriles into the corresponding amides and acids proceed with excellent selectivities under very mild conditions. So far, a large number of nitrile-hydrolyzing microorganisms have been isolated (for reviews of nitrile biodegradations see [11–13]), and they have been reported to catalyze a direct transformation of nitrile into an acid through a nitrilase and/or a hydration reaction of a nitrile via a nitrile hydratase followed by a hydrolysis of the amide with an amidase [14,15] (Scheme 1).

In our previous studies, it has been demonstrated that *Rhodococcus* sp. AJ270 is a powerful and robust

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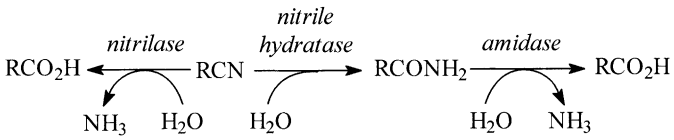


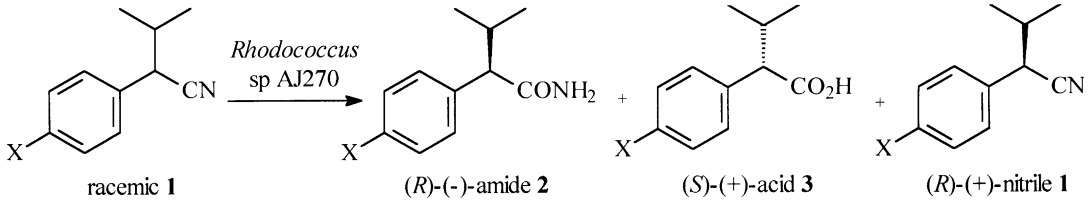
Scheme 1.

nitrile hydratase/amidase-containing microorganism. Compared with other microorganisms reported recently, it has broad activity against all types of nitriles including aromatic, heterocyclic and aliphatic ones, and both amides and acids can be obtained in high yields from appropriate nitriles [16,17]. It also shows excellent regioselectivity in hydrolyzing aromatic dinitriles and a variety of aliphatic dinitriles bearing a suitably placed second chelating moiety [18,19]. During a recent study, we have found that *Rhodococcus* sp. AJ270 was an efficient enantioselective biocatalytic system, and it was able to transform some α-substituted phenylacetonitriles into enantiopure amides and acids [20]. A similar study was also reported using other *Rhodococcus* whole-cell catalyst [21]. It was this study that led to our current investigation. Herein, we wish to report highly enantioselective biotransformations of 2-aryl-3-methybutyronitriles, a simple and convenient synthesis of enantiopure 2-aryl-3-methylbutyric acid derivatives.

2-Aryl-3-methylbutyric acids are very important intermediates of pharmaceutical and agrochemical significance. Earlier studies have shown that only the *S*-enantiomer derived compounds possess biological activities [22]. For example, a pyrethroid has been developed from the ester of (*S*)-2-(4-chlorophenyl)-3-methylbutyric acid [23], while (*S*)-2-(4-fluorophenyl)-3-methylbutyric acid was utilized as a key building block for the calcium antagonist Mibefradil [24]. Optically active 2-aryl-3-methylbutyric acids were obtained mainly from optical resolution of racemic acids by fractional crystallization [25] or preferential crystallization [26]. Asymmetric hydrogenation of 2-aryl-3-methyl-2-butenoic acids using a chiral catalyst, reported by Hayashi and Kawamura [27] and later by others [24,28], also afforded (*S*)-2-aryl-3-methylbyturic acids in high enantiomeric excesses (ee). Kinetic resolution of methyl 2-aryl-3-methylbutyric esters by horse liver esterase (HLE) has been reported to give a pair of *S*-acid and *R*-ester in the ee of 76–96% [29]. Enantioselective biotransformation of racemic 2-aryl-3-methylbutyronitriles has not been known until recently. In a note, Mitsuda and his coworkers [30] showed that a soil-derived microorganism *Pseudomonas* sp. B21C9 could resolve 2-(4-chlorophenyl)-3-methylbutyronitrile in a very dilute solution.

2. Results and discussion

In order to examine the effect of the substituent on the reaction, a series of 2-aryl-3-methylbutyronitriles **1a–e** bearing either an electron-withdrawing or electron-donating group on the aromatic ring were synthesized [31,32] and subjected to biotransformations (Scheme 2). As indicated in Table 1, the reaction rate was strongly dependent upon the steric



X = H, F, Cl, Br, MeO

Scheme 2.

Table 1
Enantioselective biotransformations of nitriles **1**

Entry	**1**	X	Reaction conditions[a]	**2**[b] (%)	ee[c] (%)	**3**[b] (%)	ee[d] (%)	**1**[b] (%)	ee[e] (%)
1	**a**	H	5d	45	>99	48	>99	–	–
2	**b**	F	6.5d	30	>99	43	>99	24	>99
3	**b**	F	3.5d (0.5 mmol)	45	>99	45	>99	–	–
4	**c**	Cl	7d	20	18	29	>99	45	29
5	**c**	Cl	7d[f]	6	>99	11	>99	79	10
6	**c**	Cl	7d (0.5 mmol)	32	76	23	>99	34	48
7	**c**	Cl	7d (0.25 mmol)	39	>99	40	>99	10	56
8	**d**	Br	7d	8	>99	16	>99	59	5
9	**e**	MeO	7d	16	0	15	>99	58	41

[a] Unless otherwise indicated, 1 mmol of the starting nitrile **1** was used in all cases.
[b] Isolated yield.
[c] Determined by chiral HPLC analysis of the amide.
[d] Determined by chiral HPLC analysis of the corresponding methyl ester.
[e] Determined by chiral HPLC analysis of the recovered nitrile.
[f] Reaction was performed in a biphasic system of aqueous phosphate buffer (25 ml) and hexane (25 ml).

nature of the *para*-substituent X. For example, while 3-methyl-2-phenylbutyronitrile **1a** was completely transformed within 5 days to give the corresponding amide and acid (Entry 1), the biotransformation of bromo- or methoxy-substituted analogs **1d** and **1e** proceeded sluggishly in a week with the recovery of more than half of the starting material (Entries 8 and 9). Having a smaller substituent such as fluoro and chloro at the *para*-position of the phenyl ring, nitriles **1b** and **1c** appeared as fairly good substrates. The conversion of 3-(4-fluorophenyl)-3-methylbutyronitrile **1b** was improved significantly when the scale of the reaction was halved (Entries 2 and 3). It should be noted that a bisphasic system of buffer and hexane did not help to improve the chemical conversion of **1c** (Entry 5).

It is noticeable that the biotransformation of all substrates tested produced (*S*)-(+)-carboxylic acids **3** in excellent optical yield (ee > 99%). However, the enantiomeric excess of amides varied dramatically depending on the substituent X and on the reaction conditions applied. Whereas, enantiopure (*R*)-(−)-amides were obtained from the parent nitrile **1a** and its fluoro and bromo-substituted analogs **1b** and **1d** (Entries 1–3 and 8), 2-(4-methoxyphenyl)-3-methylbutyramide **2e** was optically inactive (Entry 9). In the case of chloro-substituted nitrile **1c**, the enantiomeric excess of amide **2c** increased with the decrease of the reaction scale (Entries 4, 6 and 7). It is interesting to note that application of a biphasic system in biotransformation resulted in a significant improvement of enantiomeric excess of 2-(4-chlorophenyl)-3-methylbutyramide **2c**, although the conversion of **1c** was heavily retarded (Entry 5). The nitriles **1c–e** recovered from the reaction were shown to contain the *R*-enantiomer in excess, albeit in low optical yield with the exception of the fluoro-substituted nitrile **1b** which had an enantiomeric excess of >99%.

The outcomes illustrated in Table 1 demonstrated clearly that the nitrile hydratase and the amidase involved in *Rhodococcus* sp. AJ270 appeared surprisingly sensitive to the structure of the substrates. It is particularly noteworthy that even being remote from the reactive site of cyano or amido functional group, the *para*-substituent X of the phenyl ring played a role in slowing down the biotransformations, which indicated a severe steric limitation of the enzymes. Because of the steric requirement, on the other hand, the amidase in *Rhodococcus* sp. AJ270 showed a strictly *S*-enantioselectivity against 2-aryl-3-methylbutyramides **2**, excellent enantiomeric excesses being always obtained for all carboxylic acids **3**. The lower enantiomeric excess obtained for the recovered *R*-(+)-nitriles **1c–e** suggested that the nitrile hydratase in *Rhodococcus* sp. AJ270 exhibits a low *S*-enantioselectivity towards these substrates. Interestingly, the nitrile hydratase has been shown previously to present *R*-enantioselectivity against α-*n*-propyl- and α-*n*-butyl-phenylacetonitriles [20]. It seems that the nitrile hydratase involved in *Rhodococcus* sp. AJ270 has a loose enantioselectivity which is also readily affected by the substrate structures.

The formation of optically inactive amides **2c** and **2e** (Entries 4 and 9) most probably resulted from the combined effects of both *S*-selective amidase and nitrile hydratase at right conversion stage. A less efficient and moderate *S*-selective nitrile hydratase converted nitrile into amide with a moderate excess amount of *S*-enantiomer. Subsequently, the *S*-amide was transformed by the highly *S*-selective amidase into the *S*-acid, leaving unconverted amide with low enantiomeric excess. To obtain amide of high enantiopurity, it is recommended, therefore, that the reaction be monitored. Good kinetic resolution was generally effected with high conversions of both nitrile and amide, which was exemplified by the biotransformation of **1c** in low concentration (Entry 7).

3. Experimental

Both melting points, which were determined using a Reichert Kofler hot-stage apparatus, and boiling points are uncorrected. IR spectra were obtained on a Perkin-Elmer 782 instrument as liquid films or KBr discs. NMR spectra were recorded on Varian Unity 200 and Bruker AM 300 spectrometers. Chemical shifts are reported in ppm, and coupling constants are given in Hz. Mass spectra were measured on an AEI MS-50 mass spectrometer, and microanalyses were carried out by the Analytical Laboratory of the Institute.

Polarimetry was carried out using an optical activity AA-10R polarimeter, and the measurements were made at the sodium D-line with a 5 cm pathlength cell. Concentrations (*c*) are given in g per 100 ml. The enantiomeric excesses of all amides **2** were obtained with a Shimadzu LC-10AVP HPLC system using a Chiracel OD column at a flow rate 0.8 ml min^{-1}, with hexane:2-propanol [90:10] as the mobile phase. The enantiomeric excesses all acids **3** were determined from the chiral HPLC analyses of the corresponding methyl esters that were obtained from the methylation using diazomethane [33]. A Chiracel OD column with a mobile phase of hexane:2-propanol [90:10] at a flow rate 0.3 ml min^{-1} was applied for the analyses of the methyl esters of **3a** and **3e**. Methyl esters of acids **3b** and **3d** were analyzed under the same conditions but with a Chiracel OJ column. The chiral separation of recovered nitrile **1b** was carried out using a Chiracel OB with hexane:2-propanol [180:1] as the mobile phase at a flow rate 0.2 ml min^{-1}, and enantiomeric excesses of recovered nitriles **1c**, **1d** and **1e** were analyzed under the same conditions but at a flow rate 0.3 ml min^{-1}.

Nitriles were synthesized from the isopropylation of the *para*-substituted benzyl cyanides according to the literature [31,32]. Racemic amides [34] and acids [35] were obtained from chemical hydrolysis of nitriles following the literature methods. The configurations of amides and acids were determined by the comparison of the direction of optical rotation with that of authentic samples, while the stereochemistry of nitrile was obtained by comparing its optical rotation with that of nitrile derived from amides of known configuration.

3.1. General procedure for the biotransformations of nitriles

To an Erlenmeyer flask (100 ml) with a screw cap was added *Rhodococcus* sp. AJ270 cells (2 g wet weight) and the potassium phosphate buffer (0.1 M, pH 7.0, 50 ml) and the resting cells which were activated at 30°C for 0.5 h with orbital shaking. Nitrile (see Table 1) was added in one portion to the flask and the mixture was incubated at 30°C using an orbital shaker (200 rpm). The reaction, monitored by TLC, was quenched after a period of time (see Table 1) by removing the biomass through a Celite pad filtration. The resulting aqueous solution was made basic to pH 12 with aqueous NaOH (2 M). Extraction with diethyl ether gave, after drying ($MgSO_4$) and concentration, the amide and unconverted nitrile. Separation of amide and nitrile was effected by column chromatography. The aqueous solution was then acidified using aqueous HCl (2 M) to pH 2 and extracted with diethyl ether. Acid was obtained after removal of the solvent. All products were characterized by their spectra data and comparison of the melting points and optical rotary power with the known compounds, which are listed hereafter, or by full characterization.

3.1.1. Enzymatic hydrolysis of (±)-3-methyl-2-phenylbutyronitrile **1a** *[31,32]*

(*R*)-(−)-3-methyl-2-phenylbutyramide **2a**: 5d (45%) $[\alpha]_D^{25}$ − 53.1 (*c* 0.64, CH_3OH) (ref. [36] $[\alpha]_D^{24}$ + 56 (EtOH), (*S*)-(−)-3-methyl-2-phenylbutyramide), ee > 99% (chiral HPLC). Mp: 139.5–141.5°C (ref. [36] 141–142°C, (*S*)-(−)-3-methyl-2-phenylbutyramide); ν_{max} (KBr)/cm^{-1} 3406, 3191 (NH_2), 1653 (C=O);

δ_H 7.29 (s, 5H, ArH), 6.90 (br s, 1H, N*H*H), 5.87 (br s, 1H, NH*H*), 2.99 (d, $J = 9.8$, 1H, CH), 2.37–2.55 (m, 1H, CH), 1.11 (d, $J = 6.3$, 3H, CH_3), 0.76 (d, $J = 6.5$, 3H, CH_3).

(*S*)-(+)-3-methyl-2-phenylbutyric acid **3a**: 5d (48%) $[\alpha]_D^{25} + 78.4$ (*c* 2.5, $CHCl_3$) (ref. [36] $[\alpha]_D^{25} + 62.5$ ($CHCl_3$)), ee > 99% (chiral HPLC on the corresponding methyl ester), Mp: 41–43°C (ref. [37] 50.5–51.5°C); ν_{max} (KBr)/cm^{-1} 2800–3400 (COOH), 1706 (C=O); δ_H 7.32–7.39 (m, 5H, ArH), 7.12 (br s, 1H, COOH), 3.20 (d, $J = 10.6$, 1H, CH), 2.32–2.45 (m, 1H, CH), 1.13 (d, $J = 6.5$, 3H, CH_3), 0.76 (d, $J = 6.5$, 3H, CH_3).

3.1.2. Enzymatic hydrolysis of (±)-2-(4-fluorophenyl)-3-methylbutyronitrile **1b** *[37]*

(*R*)-(−)-2-(4-fluorophenyl)-3-methylbutyramide **2b**: 6.5d (30%) $[\alpha]_D^{25} - 41.6$ (*c* 1.30, CH_3OH), ee > 99% (chiral HPLC). Mp: 129–129.5°C; found C, 67.50; H, 7.07; N, 6.80. $C_{11}H_{14}FNO$ requires: C, 67.67; H, 7.23; N, 7.17; ν_{max} (KBr)/cm^{-1} 3410, 3204 (NH_2), 1657 (C=O); δ_H 7.29–7.35 (m, 2H, ArH), 7.02 (t, $J = 8.6$, 2H, ArH), 6.29 (br s, 2H, NH_2), 3.00 (d, $J = 10.3$, 1H, CH), 2.32–2.40 (m, 1H, CH), 1.08 (d, $J = 6.5$, 3H, CH_3), 0.73 (d, $J = 6.6$, 3H, CH_3); *m/z* (EI) 195 (M^+, 15%), 153 (57), 151 (36), 109 (100).

(*S*)-(+)-2-(4-fluorophenyl)-3-methylbutyric acid **3b**: 6.5d (43%) $[\alpha]_D^{25} + 48.6$ (*c* 4.20, $CHCl_3$) (ref. [24] $[\alpha]_D^{20} + 50.7$ (*c* 1, $CHCl_3$)], ee > 99% (chiral HPLC on the corresponding methyl ester). Mp: 48.5–50°C (ref. [24] 55–57°C); ν_{max} (KBr)/cm^{-1} 2500–3300 (COOH), 1706 (C=O); δ_H 8.60 (br s, 1H, COOH), 7.26–7.32 (m, 2H, ArH), 7.00 (t, $J = 8.7$, 2H, ArH), 3.14 (d, $J = 10.6$, 1H, CH), 2.25–2.35 (m, 1H, CH), 1.07 (d, $J = 6.5$, 3H, CH_3), 0.70 (d, $J = 6.7$, 3H, CH_3).

(*R*)-2-(4-flurophenyl)-3-methylbutyronitrile **1b** [37]: 6.5d (24%) $[\alpha]_D^{25} + 28.6$ (*c* 2.1, $CHCl_3$), ee > 99% (chiral HPLC). ν_{max} (KBr)/cm^{-1} 2215 (CN); δ_H 7.24–7.28 (m, 2H, ArH), 7.07 (t, $J = 8.58$, 2H, ArH), 3.64 (d, $J = 6.3$, 1H, CH), 2.04–2.24 (m, 1H, CH), 1.04 (d, $J = 5.9$, 3H, CH_3), 1.02 (d, $J = 6.5$, 3H. CH_3).

3.1.3. Enzymatic hydrolysis of (±)-2-(4-chlorophenyl)-3-methylbutyronitrile **1c** *[37]*

(*R*)-(−)-2-(4-chlorophenyl)-3-methylbutyramide **2c**: 7d (39%) $[\alpha]_D^{25} - 46.0$ (*c* 1.0, CH_3OH), ee > 99% (chiral HPLC). Mp: 122–124°C; found C, 62.26; H, 6.91; N, 6.52. $C_{11}H_{14}ClNO$ requires: C, 62.41; H, 6.67; N, 6.62; ν_{max} (KBr)/cm^{-1} 3311, 3178 (NH_2), 1662 (C=O); δ_H 7.30 (s, 4H, ArH), 6.47 (br s, 1H, N*H*H), 4.75 (br s, 1H, NH*H*), 3.00 (d, $J = 10.1$, 1H, CH), 2.30–2.45 (m, 1H, CH), 1.08 (d, $J = 6.4$, 3H, CH_3), 0.73 (d, $J = 6.6$, 3H, CH_3); *m/z* (EI) 213 (7), 211 (M^+, 22%), 171 (25), 169 (82), 167 (42), 127 (32), 125 (100).

(*S*)-(+)-2-(4-chlorophenyl)-3-methylbutyric acid **3c**: 7d (40%) $[\alpha]_D^{25} + 43.3$ (*c* 1.2, $CHCl_3$) (ref. [26] $[\alpha]_D^{20} + 48.8$ (*c* 1, $CHCl_3$)), ee > 99% (chiral HPLC on the corresponding methyl ester). Mp: 94–96°C (ref. [26] 106–107°C); ν_{max} (KBr)/cm^{-1} 2500–3300 (COOH), 1704 (C=O); δ_H 7.27 (s, 4H, ArH), 4.70 (br s, 1H, COOH), 3.17 (d, $J = 10.5$, 1H, CH), 2.27–2.35 (m, 1H, CH), 1.09 (d, $J = 6.5$, 3H, CH_3), 0.73 (d, $J = 6.7$, 3H, CH_3).

(*R*)-2-(4-chlorophenyl)-3-methylbutyronitrile **1c** [37]: 7d (10%) $[\alpha]_D^{25}$ 0 (*c* 0.5, $CHCl_3$), ee 56% (chiral HPLC); ν_{max}((KBr)/cm^{-1} 2221 (CN); δ_H 7.37 (d, $J = 8.4$, 2H, ArH), 7.26 (d, $J = 8.4$, 2H, ArH), 3.66 (d, $J = 6.2$, 1H, CH), 2.11–2.13 (m, 1H, CH), 1.07 (d, $J = 6.8$, 3H, CH_3), 1.04 (d, $J = 6.7$, ArH).

3.1.4. Enzymatic hydrolysis of (±)-2-(4-bromophenyl)-3-methylbutyronitrile **1d** *[37]*

(*R*)-(−)-2-(4-bromophenyl)-3-methylbutyramide **2d**: 7d (8%) $[\alpha]_D^{25} - 17.4$ (*c* 1.15, CH_3OH), ee > 99% (chiral HPLC). Mp: 132–134°C; found C, 51.65; H, 5.48; N, 5.38. $C_{11}H_{14}BrNO$ requires: C, 51.58; H, 5.51; N, 5.38; ν_{max} (KBr)/cm^{-1} 3396, 3196 (NH_2), 1652 (C=O); δ_H 7.47 (d, $J = 8.2$, 2H, ArH), 7.27 (d, $J = 7.6$, 2H, ArH), 6.47 (br s, 1H, N*H*H), 6.03 (br s, 1H, NH*H*), 2.94 (d, $J = 10.3$, 1H, CH), 2.33–2.41 (m, 1H, CH), 1.10 (d, $J = 6.4$, 3H, CH_3), 0.74 (d, $J = 6.7$, 3H, CH_3); *m/z* (EI) 257 (19), 255 (M^+, 20%), 215 (67), 213 (100), 211 (38), 171 (70), 169 (71).

(*S*)-(+)-2-(4-bromophenyl)-3-methylbutyric acid **3d**: 7d (16%) $[\alpha]_D^{25} + 36.4$ (*c* 0.92, $CHCl_3$) (ref. [26] $[\alpha]_D^{23} + 40$ (*c* 1, $CHCl_3$)], ee > 99% (chiral HPLC on the corresponding methyl ester). Mp: 102.5–104.5°C (ref. [26] 108–109°C); ν_{max} (KBr)/cm^{-1} 2500–3300 (COOH), 1703 (C=O); δ_H 7.43 (d, $J = 8.3$, 2H, ArH), 7.20 (d, $J = 8.3$, 2H, ArH), 6.99 (br s, 1H, COOH), 3.12 (d, $J = 10.6$, 1H, CH), 2.24–2.32 (m, 1H, CH), 1.07 (d, $J = 6.5$, 3H, CH_3), 0.70 (d, $J = 6.7$, 3H, CH_3).

(*R*)-2-(4-bromophenyl)-3-methylbutyronitrile **1d** [37]: 7d (59%) $[\alpha]_D^{25} + 1.6$ (*c* 7.4, $CHCl_3$), ee 5% (chiral HPLC); ν_{max} (KBr)/cm^{-1} 2223 (CN); δ_H 7.51 (d, $J = 8.3$, 2H, ArH), 7.19 (d, $J = 8.3$, 2H, ArH), 3.64 (d, $J = 6.1$, 1H, CH), 2.07–2.16 (m, 1H, CH), 1.22 (d, $J = 6.7$, 3H, CH_3), 1.06 (d, $J = 6.7$, 3H, CH_3).

3.1.5. Enzymatic hydrolysis of (±)-2-(4-methoxyphenyl)-3-methylbutyronitrile **1e** *[37]*

(±)-2-(4-Methoxyphenyl)-3-methylbutyramide **2e**: 7d (16%) $[\alpha]_D^{25}$ 0 (*c* 0.92, CH_3OH), ee 0%. Mp: 149–151°C; found C, 69.37; H, 8.32; N, 6.50. $C_{12}H_{17}NO_2$ requires: C, 69.54; H, 8.27; N, 6.76; ν_{max} (KBr)/cm^{-1} 3410, 3161 (NH_2), 1682 (C=O); δ_H 7.27 (d, $J = 8.6$, 2H, ArH), 6.90 (d, $J = 8.5$, 2H, ArH), 6.28 (br s, 1H, N*H*H), 6.51 (br s, 1H, NH*H*), 3.83 (s, 3H, CH_3O), 3.00 (d, $J = 10.1$, 1H, CH), 2.37–2.44 (m, 1H, CH), 1.10 (d, $J = 6.5$, 3H, CH_3), 0.78 (d, $J = 6.7$, 3H, CH_3); *m/z* (EI) 207 (M^+, 20%), 163 (100).

(*S*)-(+)-2-(4-methoxyphenyl)-3-methylbutyric acid **3e**: 7d (15%) $[\alpha]_D^{25} + 48.0$ (*c* 1.46, $CHCl_3$) (ref. [25] $[\alpha]_D^{22} + 52.8$ (*c* 1.6, $CHCl_3$)], ee > 99% (chiral HPLC on the corresponding methyl ester). Mp: 137–139°C (ref. [25] 131–132°C); ν_{max} (KBr)/cm^{-1} 2500–3300 (COOH), 1705 (C=O); δ_H 7.24 (d, $J = 7.9$, 2H, ArH), 6.86 (d, $J = 8.7$, 2H, ArH), 4.28 (br s, 1H, COOH), 3.79 (s, 3H, CH_3O), 3.12 (d, $J = 10.6$, 1H, CH), 2.35–2.45 (m, 1H, CH), 1.08 (d, $J = 6.5$, 3H, CH_3), 0.72 (d, $J = 6.7$, 3H, CH_3).

(*R*)-2-(4-methoxyphenyl)-3-methylbutyronitrile **1e** [37]: 7d (58%) $[\alpha]_D^{25} + 12.1$ (*c* 4.3, $CHCl_3$), ee 41% (chiral HPLC); ν_{max} (KBr)/cm^{-1} 2220 (CN); δ_H 7.20 (d, $J = 8.6$, 2H, ArH), 6.91 (d, $J = 8.6$, 2H, ArH), 3.81 (s, 3H, CH_3O), 3.60 (d, $J = 6.3$, 1H, CH), 2.05–2.11 (m, 1H, CH), 1.03 (d, $J = 6.8$, 6H, $2CH_3$).

3.2. Chemical transformation of (R)-(−)-3-methyl-2-phenylbutyramide **2a**

To a suspension of (*R*)-(−)-3-methyl-2-phenylbutyramide **2a** (24 mg, 0.14 mmol, $[\alpha]_D^{25} - 53.1$ (*c* 0.64, CH_3OH), ee > 99%) in dry benzene (3 ml) under nitrogen was added freshly distilled thioyl chloride (0.2 ml, 2.7 mmol) followed by the addition of dry DMF (0.2 ml) at room temperature. After stirring at room temperature for 8 h, the mixture was poured over crushed ice and extracted with benzene (3 × 30 ml). The combined extracts were washed with saturated $NaHCO_3$ solution and then with water, and dried over with anhydrous $MgSO_4$. The crude product was chromatographed to yield (*R*)-(+)-3-methyl-2-phenylbutyronitrile **1a** (20 mg, 0.13 mmol, 93%, $[\alpha]_D^{25} - 26$ (*c* 1, $CHCl_3$), ee > 99%). The spectroscopic properties were identical to those of (±)-3-methyl-2-phenylbutyronitrile.

3.3. Chemical transformation of (R)-(−)-2-(4-chlorophenyl)-3-methylbutyramide **2c**

(*R*)-(−)-2-(4-chlorophenyl)-3-methylbutyramide **2c** (91.5 mg, 0.5 mmol, $[\alpha]_D^{25} - 7.06$ (*c* 0.85, CH_3OH), ee 19%) was dissolved in anhydrous toluene (15 ml) and refluxed with P_2O_5 (25 mg, 0.18 mmol) for 4 h. The resulting solution was diluted with ice water (10 ml) and extracted with chloroform (3 × 30 ml). The organic fractions were combined and washed with brine (10 ml) and dried over with anhydrous $MgSO_4$. The crude product was chromatographed to yield (*R*)-(+)-2-(4-chlorophenyl)-3-methylbutyronitrile **1c** (72 mg, 0.4 mmol, 83%, $[\alpha]_D^{25} + 5$ (*c* 3.6, $CHCl_3$), ee 21%). The spectroscopic properties were identical to those of (±)-2-(4-chlorophenyl)-3-methylbutyronitrile.

4. Conclusion

Our study has shown that *Rhodococcus* sp. AJ270 is a useful biocatalyst for the synthesis of some enantiopure *S*-(+)-2-aryl-3-methylbutyric acids and *R*-(+)-2-aryl-3-methylbutyramides from the hydrolysis of 2-aryl-3-methylbutyronitriles under mild conditions. The nitrile-hydrolyzing enzymes involved in this novel microorganism are very sensitive to the steric effect of the *para*-substituent on the aromatic ring. While the nitrile hydratase shows low *S*-stereoselectivity against nitriles, the amidase has a strict *S*-enantioselectivity against 2-aryl-3-methylbutyramides.

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