Chemoenzymatic Synthesis of Optically Pure 1,3,4-Oxadiazol-2(3*H*)-ones with Acaricidal Activity

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Both enantiomers of 5-[1-(4-isopropoxy)phenyl]ethyl-3-(2-chlorophenyl)-1,3,4-oxadiazol-2(3*H*)-one (**1a**), a new compound with high ovicidal activity against *Tetranychus urticae* (Koch), were synthesized starting from enzymatically prepared optically pure 2-[4-(isopropoxy)phenyl]propanoic acid (**3**). Laboratory tests have shown that the biological activity of the *R* isomer is at least 400-fold higher than that of the *S* form.

Keywords: *Enzymatic hydrolysis; enantiomer resolution; acaricidal activity*

INTRODUCTION

The advantages of using stereochemically pure compounds have been recognized for many years, first for pharmaceuticals and later for agrochemicals (Tombo and Belluš, 1991). If a promising crop protection compound is a racemate or a diastereoisomeric mixture, one important step of its optimization process should be the synthesis of enantiomerically pure isomers for testing purposes. The aim is to find single isomers that prove to be biologically superior to the racemate in terms of reduced rate of application, increased selectivity against the target organism, and optimal ecological profile (Bianchi et al., 1991, 1992).

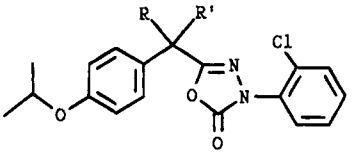
However, due to practical limitations in the synthesis of optically pure compounds, few substances, mainly of natural origin, have been developed as single stereoisomers.

In recent years, stereospecific synthesis based on biotransformation has proved to offer significant advantages over classical chemical methods involving resolution of racemates, chiral pool templates, and asymmetric synthetic reagents (Faber and Franssen, 1993; Margolin, 1993).

Hydrolytic enzymes such as lipases and proteases appear to be one of the most attractive classes of biocatalysts for the organic chemist, since they do not require any added cofactor, they are commercially available at a cheap price, and they display high stereoselectivity and regioselectivity on a broad range of substrates (Klibanov, 1990).

Recently, a new series of 3-aryl-5-(arylalkyl)-1,3,4-oxa(thia)diazol-2(3*H*)-ones with miticidal activity has been reported (Bettarini et al., 1994). Within this class, (*R,S*)-3-(2-chlorophenyl)-5-[1-[(4-isopropoxy)phenyl]ethyl]-1,3,4-oxadiazol-2(3*H*)-one (**1a**) turned out to be one of the most active compounds (Figure 1).

The present work describes the synthesis of both enantiomers of **1a** and their biological activity against eggs of *Tetranychus urticae* (Koch). The key chiral starting material for the synthesis was (*R*)- or (*S*)-2-[4-(isopropoxy)phenyl]propanoic acid (**3**), obtained through the stereoselective enzymatic hydrolysis of the corresponding methyl ester **2**.



(R,S)-1a-d

Figure 1. New acaricidal 1,3,4-oxadiazol-2(3*H*)-ones: **1a** (R = Me, R′ = H); **1b** (R = R′ = H); **1c** (R = R′ = Me); **1d** (R = Et, R′ = H).

MATERIALS AND METHODS

Apparatus. ^{1}H-NMR spectra were recorded in $CDCl_3$ solution with $SiMe_4$ as internal standard, on a Bruker AC 200 instrument. All enzymatic reactions were performed with a Methrom pH-stat. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Reagents. All organic chemicals used were purchased from Fluka Chemie. Lipase from *Candida cylindracea* and protease from *Aspergillus oryzae* were from Sigma Chemical Co. Carboxylesterase NP from *Bacillus subtilis* was purchased from International Bio-Synthetics BV, The Netherlands; lipase from *Mucor mehiei* was from NOVO Nordisk A/S, Denmark.

Procedure. *Enzymatic Hydrolysis of (R,S)-2-[4-(Isopropoxy)phenyl]propanoic Acid Methyl Ester (2) with Lipase from C. cylindracea.* The following procedure is representative. To a magnetically stirred suspension of (*R,S*)-**2** (2 g, 9 mmol) in 0.1 M phosphate buffer, pH 7 (50 mL), at 30 °C was added lipase from *C. cylindracea* (2 g). The pH was kept constant at 7 by addition of 0.1 N aqueous sodium hydroxide. Aliquots (1 μL) were periodically withdrawn and analyzed by HPLC. The hydrolysis was stopped at 50% of conversion (7 h), and the reaction mixture was extracted with *n*-hexane. The organic layer was dried (Na_2SO_4) and evaporated to dryness, affording the ester (*R*)-(+)-**2** (0.95 g, 95%): $[\alpha]^{25}_D = +70.0°$ (*c* 1, $CHCl_3$), ee >95% ; ^{1}H NMR (δ_H) 1.35 (3H, d), 1.45 (3H, d), 2.5 (3H, s), 3.6 (1 H, q), 4.5 (1H, q), 6.8 (2H, d) and 7.25 (2H, d). The aqueous phase was then acidified with 0.1 N HCl and extracted with ethyl acetate. The organic layer was dried and evaporated under vacuum, giving the acid (*S*)-(+)-**3** (0.85 g, 91%): $[\alpha]^{25}_D = +47.5°$ (*c* 1, $CHCl_3$), ee >95%; ^{1}H NMR (δ_H) 1.35 (3H, d), 1.45 (3H, d), 3.6 (1H, q), 4.5 (1H, m), 6.8 (2H, d), and 7.3 (2H, d).

Enzymatic Hydrolysis of (R,S)-2-[4-(Isopropoxy)phenyl]propanoic Acid Methyl Ester (2) with Protease from A. oryzae. The same procedure described above was used, starting from (*R,S*)-**2** (1.5 g, 6.75 mmol) and 2 g of protease from *A. oryzae*.

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The reaction was stopped at 38% of conversion, and the products were isolated as described before.

The purification gave the acid (*R*)-(−)-**3** (0.49 g, 93%) with $[\alpha]^{25}_D = -45.2°$ (*c* 1, $CHCl_3$), ee 94%, and the ester (*S*)-(−)-**2** (0.86, 91%) with $[\alpha]^{25}_D = -42.0°$ (*c* 1, $CHCl_3$), ee 57%.

Synthesis of (S)-2-[4-(Isopropoxy)phenyl]-N'-(2-chlorophenyl)-propanohydrazide] (5). A solution of the acid (*R*)-(−)-**3** (0.2 g, 0.96 mmol) in anhydrous THF (5 mL) was slowly added to a stirred suspension of 1,1'-carbonyldiimidazole (0.156 g, 0.96 mmol) in anhydrous THF (5 mL) under nitrogen atmosphere. The mixture was stirred at 25 °C for 1 h and added to a suspension of 2-chlorophenylhydrazine hydrochloride (0.172 g, 0.96 mmol) and triethylamine (0.14 mL, 0.96 mmol) in anhydrous THF (10 mL) under nitrogen atmosphere. The reaction was heated under reflux for 24 h and the solvent evaporated under vacuum. The residue was dissolved with a mixture of ethyl ether and water 1/1 (v/v) (50 mL), and the separated aqueous phase was extracted with ether (2 × 10 mL). The combined ethereal layers were washed with brine and dried with Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by chromatography on silica gel using hexane/ethyl acetate 75/25 (v/v) as eluant to give (*R*)-**5** (0.25 g, 78%): ^1H NMR (δ_H) 1.33 (6H, d), 1.53 (3H, d), 3.60 (1H, q), 4.45−4.6 (1H, m), 6.40−7.30 (10H, m).

The hydrazide (*S*)-**5** (0.235 g, 74%) was obtained by starting from (*S*)-(+)-**3** (0.2 g, 0.96 mmol) using the above described procedure.

Synthesis of (R)-3-(2-Chlorophenyl)-5-[1-[(4-isopropoxy)-phenyl]ethyl]-1,3,4-oxadiazol-2(3H)-one (1a). A solution of (*R*)-**5** (0.2 g, 0.60 mmol) in anhydrous toluene (5 mL) was slowly added with stirring to trichloromethyl chloroformate (0.15 mL, 1.2 mmol) at 25 °C under nitrogen atmosphere. The reaction was heated at 90 °C for 3 h, cooled to 25 °C, and then poured into ice−water. The reaction mixture was extracted with ethyl ether (3 × 15 mL). The combined organic layers were washed with brine and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by chromatography on silica gel using hexane/ethyl acetate 75:25 (v/v), affording (*R*)-(−)-**1a** (0.19 g, 88%): $[\alpha]^{25}_D = -44.6°$ (*c* 1, $CHCl_3$); ee 92%; ^1H NMR (δ_H) 1.31 (6H, d), 1.55 (3H, d), 4.10 (1H, q), 4.42−4.60 (1H, m), 6.80−6.90 (2H, m), 7.2−7.55 (6H, m); MS, *m/z* (%) 358 (M^+, 7), 316 (7), 121 (100); IR (Nujol) $\nu = 1790$ cm^{-1}.

(*S*)-(+)-**1a** (0.20 g, 90%), $[\alpha]^{25}_D = +45.1°$ (*c* 1, $CHCl_3$), ee 95%, was obtained by starting from the hydrazide (*S*)-**5** (0.2 g, 0.6 mmol), following the same procedure.

Optical Purity Determination. The optical purity of **2** was determined by HPLC analysis using a chiral column Daicel Chiralcel OB (Daicel Chemical Co.), with eluent hexane/2-propanol 95:5 (v/v) and flow rate 0.8 mL/min; readings were made at 220 nm. The retention times were 7.3 min for the *S* isomer and 9.6 min for the *R* isomer.

The enantiomeric excess of the acid **3** was measured by HPLC after chemical transformation into the corresponding methyl ester **2** by using diazomethane. The optical purity of **1a** was determined using the chiral column Daicel Chiralcel OC, with eluent hexane/2-propanol 9:1 (v/v) and flow rate 0.8 mL/min. The retention times were 14.7 min for the *S* isomer and 17.5 min for the *R* isomer.

Determination of Absolute Configuration of 2-[4-(Isopropoxy)phenyl]propanoic Acid (3). The acid (*R*)-(−)-**3** (0.35 g, 1.68 mmol, ee > 95%) was dissolved in acetic acid (10 mL). An aqueous solution of hydrobromic acid (48% w/v, 8 mL) was added, and the resulting solution was refluxed for 5 h. After dilution with water (15 mL), the reaction was extracted with methyl isobutyl ketone. The organic layer was dried (Na_2SO_4) and the solvent evaporated off under reduced pressure. Chromatography on silica gel with dichloromethane-methanol 95/5 (v/v) as eluant afforded (*R*)-(−)-2-(4-hydroxyphenyl)-propanoic acid (0.2 g, 71%) with $[\alpha]^{25}_D = -70.0°$ (*c* 1, EtOH); ee >95% [lit. (Nederl and Würgau, 1959) $[\alpha]^{25}_D = -71.7°$ (*c* 1, EtOH)]; ^1H NMR (δ_H) 1.45 (3H, d), 3.6 (1H, q), 6.8 (2H, d), and 7.2 (2H, d).

Ovicidal Activity against T. urticae (Koch). Leaf disks, 2.5 cm in diameter, were cut from bean leaves with egg deposit (80−100) laid during the previous 24 h. Two disks for each dose rate were dipped into water/acetone solution 9/1 (v/v) containing the compound under testing and Tween 20 (0.5 g/L). Disks treated with water/acetone solution were used as controls. Treated disks were placed on moistened cotton in open Petri dishes and kept at 25 °C and 60% relative humidity. The number of unhatched eggs was recorded 7 days after treatment, and the mortality was calculated using Abbott's correction for any spontaneous failure to emerge observed in the control. Dose−mortality responses were analyzed by probit analysis to obtain LC_{85} values.

Table 1. Hydrolase Catalyzed Resolution of (*R,S*)-2

		product **3**		
enzyme	conv (%)	$[\alpha]^{25}_D$[a]	ee (%)[b]	conf[c]
lipase from *C. cylindracea*	50	+47.6	>95	*S*
lipase from *M. mehiei*	30	+38.5	80	*S*
carboxylesterase from *B. subtilis*	48	+43.2	89	*S*
protease from *A. oryzae*	38	−45.2	94	*R*

[a] *C* = 1, $CHCl_3$. [b] Measured by HPLC using a Daicel Chiralcel OB chiral column. [c] Assigned by correlation with (*S*)-2-(4-hydroxyphenyl)propanoic acid.

RESULTS AND DISCUSSION

The racemic methyl ester **2** was synthesized by starting from 4-hydroxypropiophenone by O-alkylation with 2-bromopropane and reaction with iodine in trimethyl orthoformate, according to the literature method (Yamauchi et al., 1988). The hydrolysis of the racemic **2** was attempted by using a series of commercially available hydrolytic enzymes belonging to the classes of lipases, proteases, and esterases.

The hydrolytic reactions were carried out in a stirred emulsion of the oily substrate in phosphate buffer, pH 7 at 30 °C. The pH was kept constant by addition of sodium hydroxide using a pH-stat.

The results obtained in the hydrolysis of (*R,S*)-**2** are summarized in Table 1 and exemplified in Figure 2 for the lipase from *C. cylindracea*.

Besides the enzymes listed in Table 1, lipases from porcine pancreas and *Pseudomonas cepacia* and protease from *Bacillus* were tested, proving to be virtually inactive.

As shown in Table 1 the best results in terms of stereoselectivity were obtained using the lipase from *C. cylindracea* and the protease from *A. oryzae*. The two enzymes displayed an opposite enantiodiscrimination, affording the optically pure acid **3** in the *S* and *R* forms, respectively. This allowed us to synthesize both enantiomers of compound **1a** directly starting from the acid **3**, overcoming the chemical manipulation of the racemization-prone unreacted ester **2**.

The enantiomeric excess of optically active **3** was determined by HPLC after transformation into the corresponding methyl ester using diazomethane.

The absolute configuration of **3** was assigned by chemical correlation with the known (*S*)-2-(4-hydroxyphenyl)propanoic acid (Nederl and Würgau, 1959), after cleavage of the isopropyl ether moiety by using aqueous hydrobromic and acetic acids (Simpson, 1963), as described under Materials and Methods. The enantiodiscrimination displayed by the lipase from *C. cylindracea* was in agreement with the literature data reported for the same enzyme in the hydrolysis of differently substituted aryl propanoate derivatives (Battistel et al., 1991).

Enzymatically prepared (*R*)- and (*S*)-**3** were then used in the synthesis of optically pure **1a** (Figure 3). Since our effort to synthesize the chloride derivative of pure

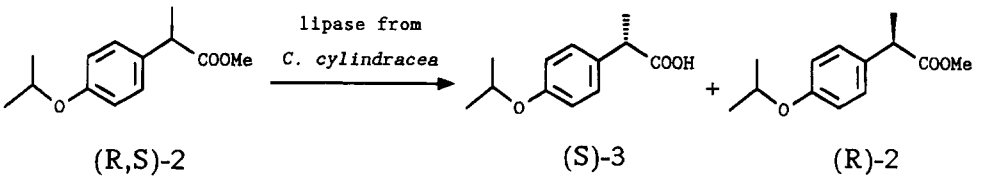

Figure 2. Enzymatic hydrolysis of racemic methyl 2-[4-(isopropoxy)phenyl]propanoate (**2**).

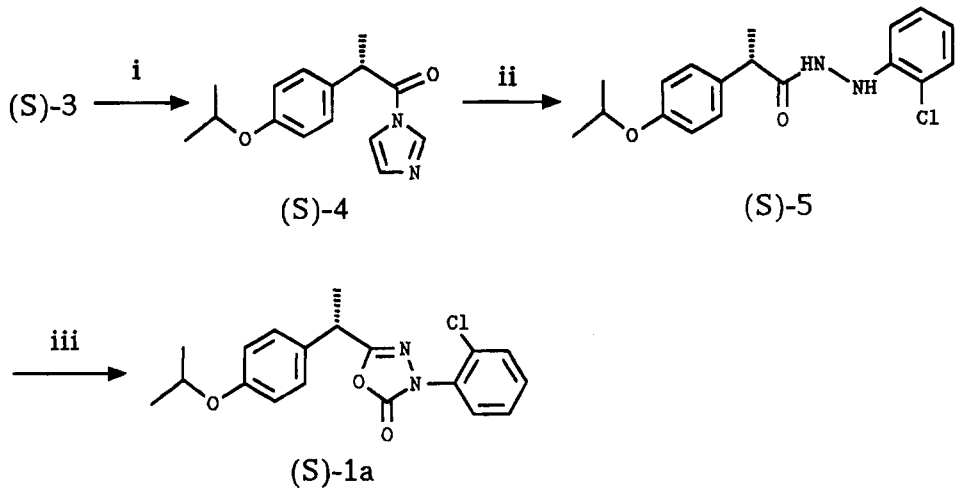

Figure 3. Synthetic route to optically active **1a**: (i) carbonyldiimidazole; (ii) 2-chlorophenylhydrazine; (iii) trichloromethyl chloroformate.

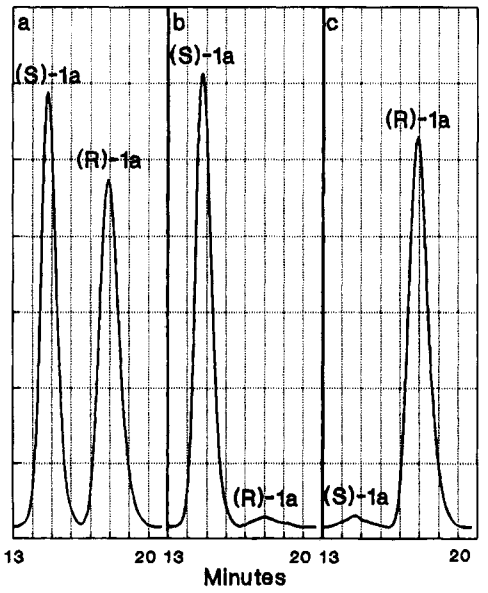

Figure 4. HPLC determination of optical purity of **1a** by a Daicel Chiralcell OC column: (a) racemic **1a**; (b) enzymatically prepared (*S*)-**1a**; (c) enzymatically prepared (*R*)-**1a**.

enantiomers of **3** resulted in the partial racemization of the final product, the activation of the carboxylic moiety was carried out by transformation into the corresponding carbonylimidazole derivative **4.** This compound was directly converted into the 2-chlorohydrazide **5** without isolation of the intermediate **4**. A treatment of **5** with trichloromethyl chloroformate yielded the final product **1a**.

The enantiomeric excess of the resulting optically active **1a** was determined by HPLC using a chiral stationary phase, as shown in Figure 4, confirming that the above described synthetic pathway proceeds in smooth conditions, without affecting the chiral center of **3**. In this way, both (*R*)- and (*S*)-**1a** were obtained with optical purity higher than 94%.

Table 2. Relative Ovicidal Potency of 1,3,4-Oxadiazol-2(3*H*)-ones 1a–d

tested compound	LC_{85}[a] (mg/L)	rel ovicidal potency
(*R,S*)-**1a**	0.24	1700
(*R*)-**1a**	0.1	4000
(*S*)-**1a**	≥40	≤10
1b	4	100
1c	67	6
(*R,S*)-**1d**	>400	<1

[a] Two replicates for each dose rate.

The relatival ovicidal potencies against *T. urticae* for the racemic and the resolved enantiomers of **1a**, the α-unsubstituted compound **1b,** the α-dimethyl compound **1c**, and the racemic α-ethyl derivative **1d** (Bettarini et al., 1994) are reported in Table 2. The *R* isomer of **1a** turned out to be 40-fold more active than **1b** and at least 400-fold more effective than the *S* isomer. It is noteworthy to point out that the small amount of (*R*)-**1a** (2%) present in the 95% optically pure *S* form (Figure 4) should also contribute to the bioactivity of the sample. However, the activity observed for it ($LC_{85} \geq 40$) was lower than expected ($LC_{85} \leq 5$) on the basis of the (*R*)-**1a** content, thus suggesting a possible antagonistic effect of the (*S*) isomer. According to that reported for similar series of α-alkyl compounds, the dimethyl derivative **1c** was in the activity range of the poorly active enantiomer (Smith et al., 1952).

In general, for a compound with pesticidal activity a strong difference in the biological response of the two optical isomers is likely due to a different three-dimensional fitting with the receptor site and/or a detoxifying enzyme. Actually, the increase in activity brought about by the introduction of a methyl group in the benzylic position may also be attributable to an increased resistance of the molecule to metabolic degradation. In such a case, the role of the methyl group of the active isomer should be mostly to hinder the interaction with the degrading enzyme (e.g., an oxidase) (Ariens, 1988). However, the almost complete loss of

activity of the α-ethyl derivative **1d** does not support this hypothesis. On the basis of the reported data, we suggest that the molecular interaction with the receptor site plays a dominant role in determining the bioactivity of the tested compounds.

In conclusion, the stereoselective enzymatic hydrolysis of **2** provided a feasible method to synthesize the optically pure enantiomers of **1a** in quantities sufficient for biological tests. Scaling-up experiments and studies on the recycle of the biocatalyst are currently in progress.

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