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Preparation of Chiral 2,4-Dimethylglutaric Acid Monoesters from the Racemic Diesters by means of Microbes and Microbial Esterases**

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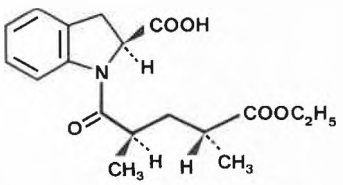
Abstract: After an extensive screening, some specialized methylotrophs as well as a *Streptomyces* strain were found to be useful sources of esterases catalyzing the enantioselective hydrolysis of racemic 2,4-dimethylglutaric acid dimethyl ester (**3**) and diethyl ester (**4**) to the corresponding optically active monoesters **5** or **9** and **7** or **11**, respectively.

The monoethyl ester of (2*R*, 4*R*)-2,4-dimethylglutaric acid (**7**) is a key chiral building block for the synthesis of the 1-glutarylindoline-2-carboxylic acid derivative **1**[1], a potent angiotensine converting enzyme (ACE) inhibitor.



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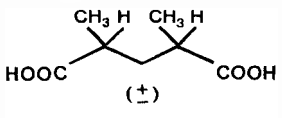
According to the literature[1], racemic 2,4-dimethylglutaric acid (**2**) can be resolved, in moderate yield, as its (+)-2-methylbenzylamine salt. Ethanolysis of the corresponding (2*R*, 4*R*)-anhydride leads to the (2*R*, 4*R*)-monoethyl ester **7**. It is also known[2], that the hydrolysis of the racemic 2,4-dimethylglutaric acid dimethyl ester **3** by whole cells of *Gliocladium roseum* afforded, by terminating the reaction at 50% turnover, the (2*R*, 4*R*)-monomethyl ester **5** with 68% *ee*. In order to obtain an *ee* > 98%, the enriched compound was subsequently esterified with diazomethane and once again hydrolyzed with *Gl. roseum*.

As there is no report of an enzymatic or microbial procedure for the preparation of the (2*R*, 4*R*)-monoethyl ester **7**, we have screened a total of 100 microorganisms from strain collections (methylotrophs), isolates from sewage treatment plants (methylotrophs and others), defined biomass from industrial processes, and soil isolates (*Actinomycetes*) for the presence of esterases catalyzing the enantioselective hydrolysis of 2,4-dimethylglutaric acid diesters.

The methylotrophic collection strains, as well as the isolates from sewage sludge, were tested for growth at 28 °C and 250



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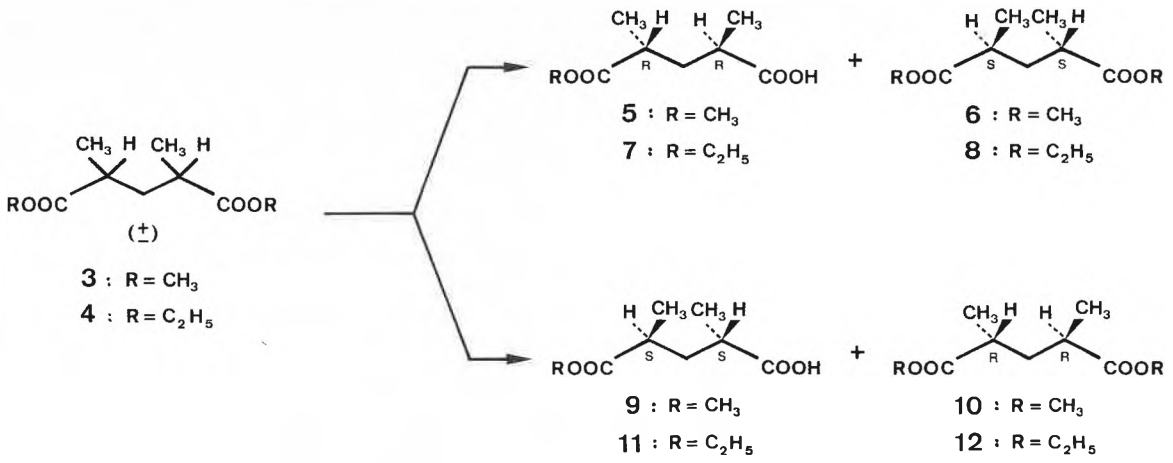

rpm in 50 mL shake flasks containing 20 mL of mineral medium MV7[3] with 1–2 g/L of racemic 2,4-dimethylglutaric acid dimethyl ester **3** or diethyl ester **4**[4], respectively, as the sole source of carbon and energy.

Thus the first selection principle was the utilization of methanol and ethanol cleaved from the substrates by action of esterases. In the case of growth, the remaining products in the fermentation broth were analyzed by capillary gas chromatography (CGC)[5].

This approach allowed the selection of 5 strains for further evaluation: *Pseudomonas* M 27 (DSM 1339, methylotroph), Isolate K1 (methylotroph), Isolate EE-9 (ethanol utilizer), Isolate EE-210 (methylotroph), and *Pseudomonas* DMF 5/8 (methylotroph, dimethylformamide utilizer[6]).

The *Actinomycetes* (*Nocardia mediterranei* and *Streptomyces* sp.) were grown on industrial fermentation media (e.g.: medium 151b[7]) and the screening for esterases was performed with 0.2–0.5 g of washed wet resting cells in 20 mL of phosphate buffer containing 1–2 g/L of diester **3** or **4**, respectively. Using this approach, *Streptomyces* R1186 was selected for a detailed evaluation. In addition, two strains of *Gliocladium roseum* (DSM 1165 = ATCC 10521 and DSM 62726) were grown according to[8] and tested as described above for the *Actinomycetes*.

This preliminary screening for substrate specific hydrolytic enzyme activities was followed by an investigation of the enantioselectivity of the individual microbial hydrolytic processes. The reactions were carried out under aerobic conditions at 28 °C and 250 rpm in 500 mL shake flasks containing 1–5 g/L of wet resting cells in 100 mL of phosphate buffer and 10–20 mmol/L of racemic diester, suspended in the aqueous phase. The turnover was determined by CGC[5]. The reaction mixture was acidified (pH 2) with 2N sulfuric acid, extracted with diethyl ether, chromatographed, and distilled bulb to bulb. The *ee*-values were calculated by comparing the $[\alpha]_D^{25}$-values of the isolated products with the corresponding values of the optically pure references[9].

The optimization of the reaction conditions and the comparison of the selectivities of the individual microbial systems was performed using the *E*-values (enantiomeric ratio) computed as described by *Chen* et al.[10]. The experimental results are summarized in Tables 1 and 2.

The observed enantioselectivities are moderate, as is usually the case for microbial esterases with a broad substrate specificity. *Streptomyces* R 1186 (run 5, Table 1) is the most suitable strain for the preparation of (2*R*,4*R*)-2,4-dimethylglutaric acid monoethyl ester **7**. As calculated from the *E*-value 8.8, one recycling step would lead to an *ee* > 90%. The strain *Streptomyces* R 1186, however, can not be used for the preparation of the monomethyl ester **5**, as the primary hydrolysis product is further degraded by the microorganism (run 6, Table 2).

Strain *Pseudomonas* M 27 and Isolate EE-210 show opposite enantioselectivities with respect to the hydrolysis of dimethyl (**3**) and diethyl (**4**) esters (runs 1, 4, Table 1 and runs 1, 5, Table 2).

The two strains of *Gliocladium roseum* show a higher selectivity with respect to the dimethyl ester **3** in comparison to the diethyl ester **4** (runs 6, 7, Table 1 and runs 7, 8, Table 2).

Pseudomonas DMF 5/8[6] has the highest *E*-value (17.5) for the hydrolysis of dimethyl ester **3**, although the subsequent partial hydrolysis of the resulting (2*R*, 4*R*)-monoester **5** puts a limit to its synthetic application as whole cells (run 3, Table 2).

For other purposes we have isolated the esterases from the isolates EE-9 and EE-210 and purified them by ammonium sulfate fractionation (EE-9), acid precipita-

Table 1. Results of microbial hydrolysis of (±)-2,4-dimethylglutaric acid diethyl ester (**4**).

run	strain	conditions pH	*T* (°C)	% monoester [a]	*ee* (%) [b]	*E*
1	*Pseudomonas* M 27	7	28	40 (**7**, *RR*)	50	4.1
2	Isolate K1	7	28	50 (**11**, *SS*)	50	4.8
3	Isolate EE-9	8	25	**40** (**11**, *SS*)	27	2.0
4	Isolate EE-210	8	25	75 (**11**, *SS*)	21	2.7
5	*Streptomyces* R 1186	8	25	48 (**7**, *RR*)	66	8.8
6	*Gl. roseum* (DSM 1165) [c]	7	28	43 (**7**, *RR*)	31	2.4
7	*Gl. roseum* (DSM 62726)	7	28	56 (**7**, *RR*)	11	1.4

[a] Determined by CGC[5]; the enriched enantiomer and the corresponding absolute configuration are in brackets. [b] *ee*-value of the isolated monoethyl ester after chromatography on silicagel and bulb to bulb distillation; the recovery of the non-hydrolyzed diester **4** is quantitative. [c] Strain DSM 1165 is identical with strain ATCC 10521.

Table 2. Results of microbial hydrolysis of (±)-2,4-dimethylglutaric acid dimethyl ester (**3**).

run	strain	conditions pH	*T* (°C)	% monoester [a]	*ee* (%) [b]	*E*
1	*Pseudomonas* M 27	7	28	35 (**9**, *SS*)	47	3.5
2	Isolate K1	7	28	54 (**9**, *SS*)	10	1.3
3	*Pseudomonas* DMF 5/8	7	37	30 (**9**, *SS*) [c]	85	17.5
4	Isolate EE-9	8	28	–	–	[d]
5	Isolate EE-210	8	28	36 (**5**, *RR*)	20	1.0
6	*Streptomyces* R 1186	8	28	–	–	[e]
7	*Gl. roseum* (DSM 1165) [f]	7	28	54 (**5**, *RR*)	43	4.0
8	*Gl. roseum* (DSM 62726)	7	28	47 (**5**, *RR*)	59	6.4
9	*Gl. roseum* [g]	7	25	50 (**5**, *RR*)	68	10.5

[a] Determined by CGC[5]; the enriched enantiomer and the corresponding absolute configuration are in brackets. [b] *ee*-value of the isolated monomethyl ester after chromatography on silicagel and bulb to bulb distillation; the recovery of the non-hydrolyzed diester **3** is quantitative. [c] Partial degradation of the resulting monoester. [d] No reaction. [e] Total degradation of product and starting material. [f] Strain DSM 1165 is identical with strain ATCC 10521. [g] Results from reference[2].

tion (EE-210), and chromatography on DEAE-sephacel. Fractions with the enriched enzyme showed a high hydrolytic activity with respect to methyl and ethyl esters of various glutaric acid derivatives. In the case of the 2,4-dimethylglutaric acid esters **3** and **4**, the isolated esterases had the same enantioselectivity as the whole cells.

The results discussed in this paper illustrate the applicability of a rational screening approach to enantioselective enzymatic reactions. Moreover, it was shown that microorganisms isolated for specific waste degradation processes can be an excellent source of enzymes for the preparation of chiral organic compounds.

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[3] O. Ghisalba, M. Küenzi, *Experientia 39* (1983) 1257.
[4] The racemic 2,4-dimethylglutaric acid diesters **3** and **4** as well as a sample of the optically pure (2*R*, 4*R*)-monoethyl ester **7** were kindly provided by Dr. R.H. Schwartz, Pharmaceuticals Division, Ciba-Geigy Co., Summit NJ (USA).
[5] OV-351/FFAP glass capillary column (20 m/0.3 mm); temperature program: 70°C to 200°C at 10°C/min; carrier gas H_2 (0.35 kg/cm^2).
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[8] J. Rosazza, P. Foss, M. Lemberger, C.J. Sih, *J. Pharm. Sci. 63* (1974) 544.
[9] Compound **5**, $[\alpha]_D^{25} = -31°$ ($CHCl_3$)[2]; compound **7**, $[\alpha]_D^{25} = -36.5°$ (*c*: 0.54, $CHCl_3$).
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