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MICROBIAL TRANSFORMATION OF (-)-VERNOLIC ACID INTO (4R,5R)-5-HYDROXY-γ-DECALACTONE

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Abstract

(-) Vernolic acid, isolated and purified from seeds of *Euphorbia lagascae* was administered to cultures of *Sporobolomyces odorus*. (4R,5R)-5-Hydroxy-γ-decalactone **1** accumulated as the main product. The configuration of the product was determined by synthesis of all four stereoisomers and comparison of spectroscopic and chromatographic data.

The isolation of 5-hydroxy-γ-decalactone **1** from cultures of *Streptomyces griseus* and the assumed biological importance as an autoregulatory factor initiated the development of synthetic routes towards all stereoisomers of this so called L-factor[2-8]. However, the autoregulatory function of this compound could not be confirmed and its biological function remained unclear. Other naturally occuring 5-hydroxy-γ-lactones were identified as flavour constituents in wine[9], sherry[10], and tobacco smoke[11] and as microbial metabolites in cultures of *Erwinia quercina*[12] and of a Nigrospora species[13]. With the isolation of 5-hydroxy-γ-heptadecalactone (Muricatacin) from seeds of *Annona muricata*[14], a compound exhibiting cytotoxic activity on tumor cell lines, this class of compounds has stimulated new interest and synthetic strategies toward this compound have been published[15,16]. As an alternative to the chemical approaches for the synthesis of optically active 5-hydroxy-γ-lactones Fronza et al.[17] presented a chemoenzymatic method with a baker's yeast catalyzed reduction of 5-keto-γ-lactones as the configuration determining step. In the present paper the direct microbial conversion of the readily available vernolic acid (12,13-epoxy-(Z)-9-octadecenoic acid) into **1** is described. The capacity of the yeast *Sporobolomyces odorus* i) to degrade long chain hydroxy acids to γ- and δ-lactones in good yields[18,19] and ii) to transform 3,4-epoxydecanoic acid into γ-decalactone[20] promoted us to employ long chain epoxy acids as lactone precursors. For this study vernolic acid, the main constituent in seed oils of several *Euphorbia* species, was used.

From seeds of *Euphorbia lagascae* vernolic acid ethyl ester was purified after extraction by transesterification and chromatographic separation. The configuration was (12R,13S) as determined by comparison of the optical rotation ($[\alpha]_D$= -0.702 +/- 0.02, c=11.05 (MeOH)) with literature data[21]. The substrate was administered to

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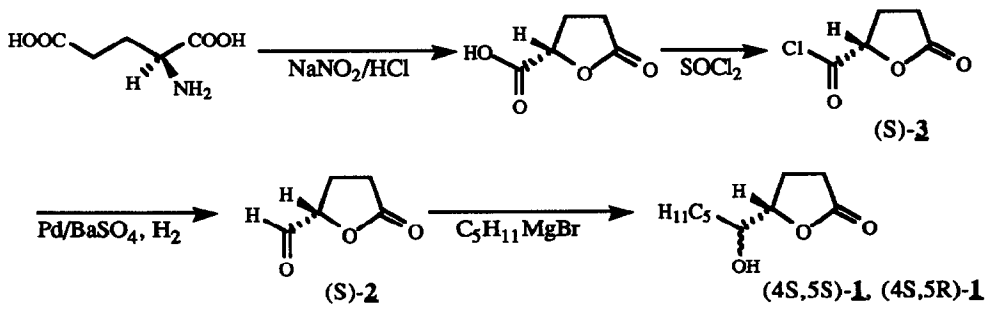
6 day old cultures of *Sp. odorus* ATCC 26697 and the metabolism was followed by GC-MS. 5-Hydroxy-γ-decalactone, identified by mass spectrometry and ^{1}H-NMR-spectroscopy[22], accumulated as the only main product. 335 mg/L was the maximum yield which corresponds to a 40% conversion of vernolic acid. As minor constituents 4,5-dihydroxydecanoic acid, 6,7-dihydroxydodecenoic acid, and 8,9-dihydroxytetradecenoic acid could be tentatively identified, based on the mass spectra of the silylated compounds[23].

In order to determine the absolute configuration, all four stereoisomers were synthesized (Scheme). According to the procedure of Doolittle et al.[24] (S)- and (R)-tetrahydro-5-oxo-2-furancarbaldehyde **2** were prepared from (S)- and (R)-glutamic acid, respectively. As determined by the gas chromatographic separation of the diastereomeric esters formed from optically pure (S)-octan-2-ol and the acid chloride **3**[25], the deamination of glutamic acid was accompanied by slight racemization. The optical purities of the acid chlorides were 92% ee ((S)-**3**) and 94.6 % ee ((R)-**3**), respectively. An inverse Grignard reaction with the aldehyde **2** and pentylmagnesiumbromide furnished a mixture of *syn*- and *anti*-**1** which could be separated by HPLC.



Scheme: Synthesis of (4S,5S)- and (4S,5R)-5-hydroxy-γ-decalactone from (S)-glutamic acid

In Figure 1 the ^{1}H-NMR of H-4 and H-5 of the biotransformation product compared with the synthesized reference compounds are shown. The *anti*-orientation of the two oxygens at C-4 and C-5 could be confirmed based on the spectral data published by Mori and Otsuka[2] and by Cooper et al.[3]. The determination of the absolute configuration was achieved by gas chromatographic separation of (4S,5S)- and (4R,5R)-**1** after conversion with (R)-Phenylethylisocyanate into the corresponding diastereoisomers (Figure 2). The configuration of the microbiologically derived product was (4R,5R) with an optical purity of 93.8 % ee.

Due to the experimental data the hydrolysis of the oxirane ring of vernolic acid occured along with an inversion of configuration at C-13. By acid catalyzed epoxide cleavage of (-)-vernolic acid an excess of (12R,13R)-dihydroxyacid was also observed[26]. However, in the case of this biotransformation an enzymatic catalysis may be assumed because the epoxide was stable under the conditions of transesterification of the seed oil. β-Oxidation of the (12R, 13R)-dihyroxyacid leads to (4R,5R)-4,5-dihydroxydecanoic acid, the immediate precursor of (4R, 5R)-**1**.

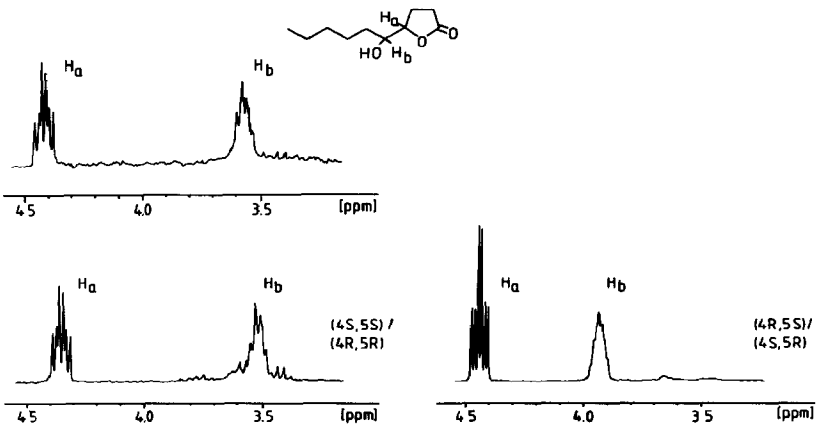


Figure 1: ^{1}H-NMR-signals of H-4 and H-5 of 1. Upper spectrum: 1 isolated from the fermentation broth; lower spectra: synthesized reference compounds.

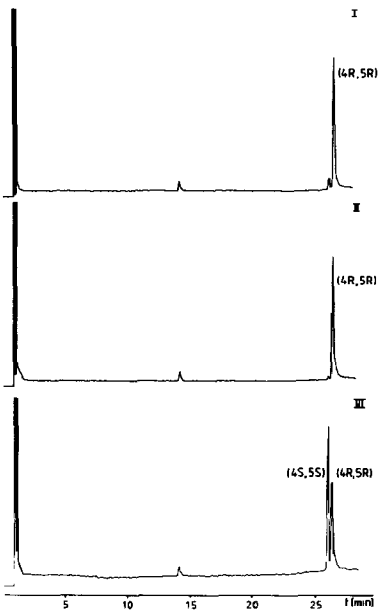


Figure 2: Chromatographic Separation of (4S,5S)- and (4R,5R)-1 after conversion with (R)-1-Phenylethylisocyanate; I: product obtained by biotransformation; II: synthesized (4R,5R)-1; III: (4S,5S)-1, coinjected with (4R,5R)-1.

This study represents an alternative method for the preparation of **1** with the drawback that only one of four possible isomers is accessible. However, the use of (+)-vernolic acid which is also naturally occuring would lead to (4S,5S)-**1**. Additionally, due to the natural occurence of further epoxy fatty acids in seeds of several plants[27] this strategy toward 5-hydroxy-γ–lactones can be expanded.

Experimental

Chemicals. All chemicals used were purchased from Fluka, Neu-Ulm (Germany) and Merck, Darmstadt (Germany). Seeds of *Euphorbia lagascae* were obtained from Prof. Röbbelen, Institut für Pflanzenbau und Pflanzenzüchtung, Universität Göttingen (Germany).

Preparation of Vernolic acid ethyl ester. 25 g seeds of *E. lagasque* were grinded in a water cooled mill for 1 min and were subsequently extracted with 150 ml pentane for 18 hours in a Soxhlet extraction apparatus. After evaporation of the solvent the yellow oil was refluxed with 50 mL 5 % sodium ethoxide in ethanol for 30 min. Ethanol was removed and the residue was acidified with 2% H_2SO_4 and extracted with 200 mL pentane/ether (1:1). The organic layer was washed with water, dried over Na_2SO_4 and concentrated. Purification of 1.0 g crude oil by liquid solid chromatography (LSC) (65 g silica gel; elution with 150 mL pentane/dichloromethane (2:1), 150 mL pentane/ether (5:1), and 150 mL ether) yielded 620 mg chemically pure vernolic acid ethyl ester ($IK_{DB\ 1}$: 2321, **EI-MS** (70 eV), m/z(rel. Int.[%]): 279 ([M-OC_2H_5]$^+$, 1.3), 41 (100), 55 (94), 67 (61), 81 (42), 95 (32), 99 (19).

Biotransformation. *Sp. odorus* ATCC 24259 was maintained on wort agar slants at 4°C. 500 mL Erlenmeyer flasks, containing 100 mL medium (per liter deionized water: 45 g sucrose, 5.0 g lactose, 3.0 g $MgSO_4 \cdot 7H_2O$, 2.5 g KH_2PO_4, 2.5 g $(NH_4)_2SO_4$, 2.5 g L-Alanine, 0.1 g $CaCl_2 \cdot 2H_2O$; pH 5.O) were inoculated. After 48 hours, 10 mL were transferred into 1L flasks containing 250 mL medium. 250 mg (=77 mmol) vernolic acid ethyl ester were added after 144 hours of cultivation. The degradation of vernolic acid was followed by GC-MS of methylated extracts prepared from 20 ml aliquots of the culture broth.

Synthesis of (4R,5R)-/ (4R/5S)-5-hydroxy-γ–decalactone. 6.2 g (89.85 mmol) solid $NaNO_2$ were added to a solution of 10 g (68.5 mmol) D-glutamic acid and 100 mL water. 41 mL 2 N HCl were added dropwise over a period of 20 min while the temperature was maintained at 15-18°C and the clear solution was stirred overnight at r.t. Water was removed in vacuo and the oily residue was extracted with hot acetone. After filtration and drying over Na_2SO_4 8.0 g (61.5 mmol) **4** could be isolated (=89.9 %). This was refluxed with 10 mL thionyl chloride for 4 hours and 6.4 g (43.1 mmol) **3** were obtained after distillation (Kp_{10}: 130-135°C) (=70 %). The optical purity was 94.4 % ee as determined by GC after formation of the diastereomeric esters with optically pure (S)-(+)-Octan-2-ol[24].

6.0 g (40.4 mmol) **3** were added to a H_2 saturated suspension of 3.0 g Pd (5% on $BaSO_4$) and toluene (distilled from sodium). H_2 was passed through the mixture for 20 hours at 50°C while the evaporation of HCl was monitored. Several spoons of Celite were added and the suspension was filtered through a Celite pad

which was subsequently washed with 200 mL dichloromethane. After removal of the solvent crude **2** (approximately 4.5 g) was dissolved in 50 mL tetrahydrofuran. Then pentyl magnesium bromide, prepared from 5.66 g (37.5 mmol) bromopentane and 0.9g (37.5 mmol) magnesium in 60 mL ether was added dropwise. After stirring for two hours at r.t. the reaction was stopped by the addition of crushed ice and 6 N HCl. The organic layer was decanted and the aqueous phase was extracted with 100 mL ether. The combined extracts were washed with 5% $NaHCO_3$ and water and then dried over Na_2SO_4. The solvent was removed in vacuo and the residue was purified chromatographically on silica. 2.9 g (15.6 mmol) of a mixture of (4R,5S)-**1** (IK_{DBWAX} : 2686) and (4R,5R)-**1** ($IK_{DB\ WAX}$: 2734) was obtained (= 41 %). Separation of the diastereoisomers was achieved by HPLC on silica.

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(22) **EI-MS** (70eV), m/z (rel. int. [%]): 186 (M^+, 2), 169 (1.1), 158 (0.7), 86 (100), 55 (39), 83 (22), 85 (21). **CI-MS** (CH_4): 169 ($(M+1-H_2O)^+$, 100), 187 ($(M+1)^+$, 13).

^{1}H-NMR (250 MHz, $CDCl_3$):

δ [ppm]		
0.827	3H, t, J=7.0 Hz	H-10
1.10-1.538	8H, m	
1.79	1H, s (br.)	-OH
1,94-2.28	2H, m	
2.38-2.65	2H, m	
3.44-3.65	1H, m	H-5
4.35	1H, dt, J=4,5 Hz, 15 Hz	H-4

(23) By electron impact ionization the characteristic fragmentation of silylated vic-diols is the C-C-cleavage between the two O-trimethylsilyl (TMS) moieties.**EI-MS** (70eV):

4,5-di-O-TMS-decanoic acid: 73 (100), 173 (86), 189 (67);

6,7-di-O-TMS-dodecenoic acid: 73 (100), 173 (94), 215 (83);

8,9-di-O-TMS-tetradecenoic acid: 73 (100), 173 (49), 243 (28);

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