synthesis of other hydroperoxide natural products is in progress.

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Supplementary Material Available: Experimental procedures, spectral characterization, and ^1H NMR spectra of all new compounds (18 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Studies on the Biosynthesis of Aliphatic Lactones in *Sporobolomyces odorus*. Conversion of (*S*)- and (*R,S*)-13-Hydroxy-(*Z,E*)-9,11-octadecadienoic Acid into Optically Pure (*R*)-δ-Decalactone

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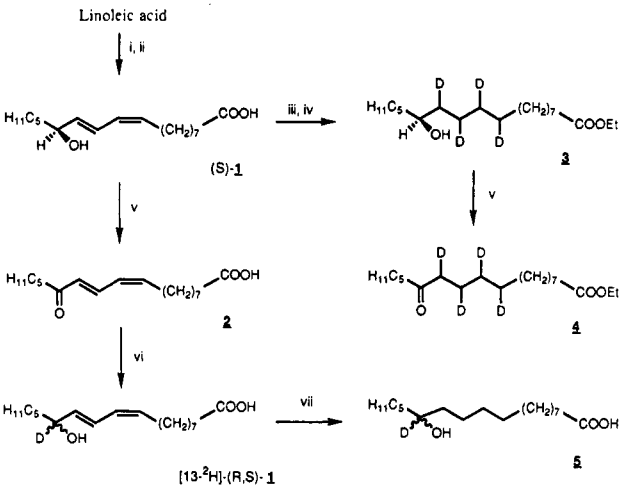
Summary: The mechanism of the biotransformations of (*S*)- and (*R,S*)-13-hydroxy-(*Z,E*)-9,11-octadecadienoic acid, **1**, into optically pure (*R*)-δ-decalactone, **12**, catalyzed by the yeast *Sporobolomyces odorus*, was studied, and an oxidation of the secondary hydroxy group followed by an enantioselective reduction of the keto acid intermediate was found to be responsible for the stereochemical outcome.

The yeast *Sporobolomyces odorus* excretes a series of aliphatic γ- and δ-lactones during growth,[1] which are known as important flavor compounds. Two main products (*R*)-γ-decalactone (>98% ee) and (*R*)-(*Z*)-6-dodecen-4-olide (84% ee), can be isolated from cultures of this organism. (*R*)-δ-**12** and (*S*)-(*Z*)-7-decen-5-olide accumulate in minor amounts and, after reaching maximum concentrations of 5 mg/L, both compounds are reabsorbed and degraded by the cells.[2] In the course of our studies on the biosynthesis of lactones in the yeast *Sp. odorus*, the transformation of [9,10,12,13-$^2H_4$]-linoleic acid into **12** could be detected. Additionally, ^{18}O labeled (*S*)-13-hydroperoxy- and 13-hydroxy-(*Z,E*)-9,11-octadecadienoic acid were generated by soybean lipoxygenase catalyzed hydroperoxidation of linoleic acid and administered to cell suspensions of *Sp. odorus*. This organism demonstrated the ability to transform both precursors into **12**.[2] These results indicate that a hydroperoxidation and a subsequent reduction are the initiating steps in the biosynthesis of (*R*)-**12**, which is formed by β-oxidation of the long-chain hydroxy acid. However, all lipoxygenases which have been isolated and characterized from plant sources or mammalian cells catalyze the hydroperoxidation of linoleic acid either stereospecifically, leading to the (*S*)-13-enantiomer (60–94% ee) or without stereospecificity.[3] Therefore, the mechanism of the conversion of enzymatically formed 13-hydroxy-(*Z,E*)-octadecadienoic acid (coriolic acid), **1**, into optically pure (*R*)-**12** remained unclear.

Recently, biotransformations of **1** into (*S*)-**12** (80% ee) with *Cladosporium suaveolens* were described.[4] The optically active lactone (78–82% ee) was also obtained when racemic coriolic acid was added to growing cultures of this organism. Studies using labeled (*S*)- (90% ee) and racemic 14-hydroxy-(*Z,E*)-10,12-nonadecadienoic acid as incubation substrates were also conducted. The degradation of both the optically active and the racemic precursor resulted first in the accumulation of (*S*)-γ-nonalactone. However, with continued fermentation the enantiomeric purity decreased, finally ending with a product mixture of predominantly (*R*)-configuration. Analysis of the chiral products by ^2H-NMR led to the hypothesis that different mechanisms are responsible for the degradation of each enantiomer of the substrate. Unequal rates in the formation and, later, in the catabolism of the products thus leads to the accumulation of optically active lactones.[4]

In this paper, stereochemical features of the transformation of (*S*)- and racemic **1** into optically pure (*R*)-**12**,

Scheme I. Synthetic Route Leading to the Precursors for the Feeding Experiments[a]



[a] Key: (i) soybean lipoxygenase;[7] (ii) $NaBH_4$; (iii) Pd/C, $^2H_2$; (iv) $EtOH/CH_3COCl$; (v) pyridinium chlorochromate;[8] (vi) NaB^2H_4; (vii) Pd/C, H_2.

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Table I. Quantitative Data of the Biotransformations Leading to δ-Decalactone

substrate	amount (μmol)	δ-decalactone amount (μmol)	yield (%)	optical purity (% ee)
(*S*)-1	360	56.5	15.7	>98
[9,10,12,13-$^2H_4$]-(*S*)-1	360	61.2	17.0	>98
[13-^2H]-(*R,S*)-1	240	36.0	15.0	>98
2	240	33.6	14.0	>98
3	360	4.7	1.3	>98
4	240	2.9	1.2	>98
5	240	8.6	3.6	>98

catalyzed by *Sp. odorus*, are presented. Chirospecific and mass spectrometric analysis of products generated from deuterium-labeled precursors revealed that an oxidation of the hydroxy group followed by an enantioselective reduction is responsible for the inversion of the configuration.

The precursors (*S*)-1, [13-^2H]-(*R,S*)-1, **2**, **3**, **4**, and **5** were prepared according to Scheme I. [9,10,12,13-$^2H_4$]-(*S*)-1 was obtained starting from [9,10,12,13-$^2H_4$]-linoleic acid.[2] Purification of the substrates was accomplished either by thin-layer or column chromatography on silica, and the structures were confirmed by EI-MS (70 eV) and ^1H-NMR. *Sp. odorus* ATCC 24259 was cultivated in 1-L Erlenmeyer flasks containing 250 mL of medium (45 g of sucrose, 5 g of lactose, 3.0 g of $MgSO_4 \cdot 7H_2O$, 2.5 g of KH_2PO_4, 2.5 g of $(NH_4)_2SO_4$, 2.5 g of L-alanine, and 0.1 g of $CaCl_2 \cdot 2H_2O$ per liter of deionized water). Substrates (240–360 μmol) were added after 120–150 h of cultivation when the concentration of the previously biosynthesized δ-lactones decreased below 0.1 mg/L. For the identification and quantitative determination of metabolites, an aliquot was taken from the culture broth, an internal standard was added, and the samples were extracted with ether. After methylation with diazomethane, the samples were subjected to GC–MS analysis. The configuration and optical purity of **12** were determined gas chromatographically after the formation of diastereomeric 5-[(*R*)-[(1-phenylethyl)-carbamoyl]oxy]decyl-*N*-butylamides.[5]

The results of the biotransformations are summarized in Table I. After administration of (*S*)-1, optically pure (*R*)-**12** could be isolated in a maximum yield of 15.7%. Additionally, 13-oxo-9,11-octadecadienoic acid (**2**) accumulated temporarily in the fermentation broth. This compound could be identified by the comparison of gas chromatographic retention time and mass spectral data with that of the synthesized substance. The result of the stereochemical analysis and the detection of **2** as an intermediate indicated that an oxidation–reduction mechanism is responsible for the inversion of the configuration. In order to confirm this hypothesis, [9,10,12,13-$^2H_4$]-(*S*)-1, [13-^2H]-(*R,S*)-1, and **2** were used as substrates. [9,10,12,13-$^2H_4$]-(*S*)-1 was converted to the lactone, which possessed two deuterium atoms as determined by MS. Therefore, a second deuterium must have been removed in addition to that located at C-9, which represents the carboxyl carbon of the lactone. The biotransformation of [13-^2H]-(*R,S*)-1 into the lactone proceeded in a similar yield and was accompanied by the complete removal of deuterium. This result provides support for the proposed oxidation and explains the loss of a second deuterium in the course of the degradation of [9,10,12,13-$^2H_4$]-(*S*)-1. In order to demonstrate the enantioselective reduction of the proposed oxidation product, **2** was fed to *Sp. odorus* in a similar manner. **12** was obtained in comparable yield, and

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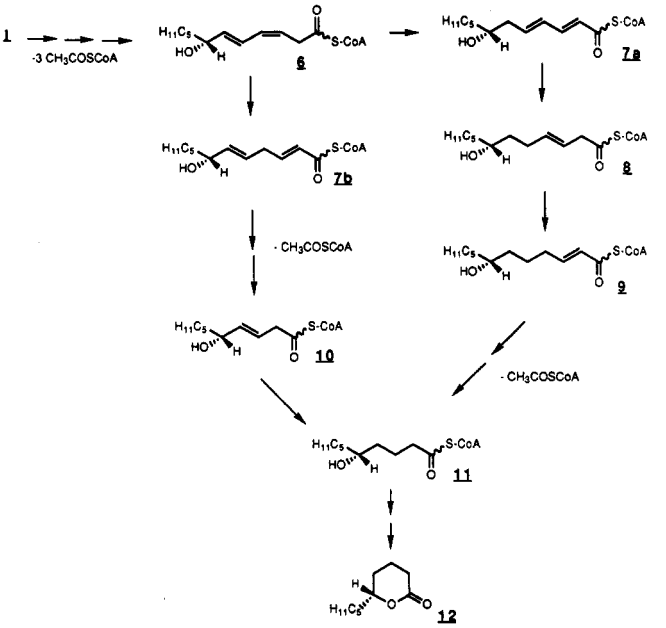
Scheme II. Proposed Degradation Pathway of Coriolic Acid Leading to δ-Decalactone



the optical purity was >98%. The results of these studies give support to the proposal that **1** is converted into optically pure **12** of opposite configuration.

In order to investigate the dependence of the presence of the diene structure on the enantioselectivity of this mechanism, the ethyl esters **3** and **4** and the acid **5** were fed to cultures of *Sp. odorus*. (Previous incubation experiments had shown that aliphatic esters were quantitatively hydrolyzed within 2–4 h.)[6] The conversions of **3** and **5** into **12** were confirmed by MS. The lactone which accumulated after the addition of **4** was not labeled, indicating that, as in the case of the incubation experiment with [13-^2H]-(*R,S*)-1, the deuterium was removed by oxidation of the secondary hydroxy group. Stereochemical analysis revealed that the lactone was produced in the same high optical purity as from the unsaturated precursors. It appears that the stereoselectivity of this enzymatic reaction is not influenced by the absence of the diene structure. However, in contrast to the stereochemical outcome, the quantitative data demonstrate a decisive influence of the diene moiety on the yield of the lactone. The maximum conversion of the saturated precursors into the lactone did not exceed 3%. Therefore, it can be concluded that the degradation of **1** leads to the formation of intermediates which are not accepted by the β-oxidation enzymes as efficiently as the saturated analogues. These results can be rationalized based on knowledge of the metabolism of unsaturated fatty acids (Scheme II). In order to explain their results after similar incubation experiments, Fronza et al. proposed an isomerization of **6** via a 1,5-hydrogen shift leading to **7a**.[9] However, (*E,Z*)- and (*E,E*)-2,4-dienoic acids are known to be reduced by a NADPH-dependent 2,4-dienoyl-CoA reductase,[10] and after isomerization of the reduction product **8**, the further metabolism of **9** is identical to that of the saturated hydroxy acids. Therefore, the high accumulation of **12** cannot be explained by this pathway. The alternative pathway is

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initiated by an enoyl-CoA isomerase catalyzed 1,3-hydrogen shift yielding **7b** and, after an additional β-oxidation cycle, 5-hydroxy-(*E*)-3-decenoyl-CoA **10**. Assuming that the 5-hydroxy group prevents a further degradation of **10** via the usual pathway, **11** can be generated by reduction of the Δ^3 double bond. This is similar to the recently described enzymatic hydrogenation of (*Z*)-5-dodecenoyl-CoA as an essential step in the metabolism of oleic acid in rat liver cells.[11] The latter pathway is supported by the fact that a 1,3-proton shift, catalyzed by enoyl-CoA isomerase, proceeds intramolecularly and by the stipulation that the CoA-group and the C2–C3 bond are rigidly fixed to the enzyme.[12]

Although further studies are required in order to fully elucidate the mechanism of the degradation of **1**, it is evident that the reactions responsible for the stereochemical outcome are independent from the presence of double bonds and are different from the proposed mechanisms previously described.[4,9,13] Additionally, the possible role of **1** in the biosynthesis of (*R*)-**12**, produced by *Sp. odorus*, was demonstrated.

Supplementary Material Available: Procedures and selected NMR, UV, and mass spectra of **1–5** (20 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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Preparation of Functionalized Dialkylzinc Reagents via an Iodine–Zinc Exchange Reaction. Highly Enantioselective Synthesis of Functionalized Secondary Alcohols

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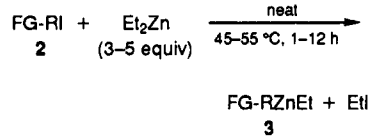
Summary: Functionalized dialkylzincs are obtained by the reaction of polyfunctional alkyl iodides with Et_2Zn in excellent yield. Their treatment with aldehydes, in the presence of the titanium catalyst **6**, affords functionalized secondary alcohols with high enantioselectivity.

Dialkylzincs (R_2Zn) are important intermediates in organic synthesis since their addition to aldehydes in the presence of various chiral catalysts allows the preparation of optically active secondary alcohols with a high level of enantioselectivity.[1-3] No general preparation[4] of R_2Zn is available, and this has limited their applications in organic synthesis. For the lower terms (R ≤ Bu) a direct insertion of zinc to an alkyl iodide followed by the distillation of R_2Zn is possible.[5] The transmetalation of lithium,[6] magnesium,[3d,7] aluminum,[8] boron,[9] and mercury[10] organometallics with zinc salts has also been frequently used for the preparation of R_2Zn. We report herein a new preparation of highly functionalized primary dialkylzincs **1** by using an iodine–zinc exchange[11] reaction (eq 1).



Thus, the treatment of an alkyl iodide, FG-RI, **2** with an excess of diethylzinc[12] (3–5 equiv) at 45–55 °C without

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