Biosynthesis of (*R*)-γ-Decanolactone in the Yeast *Sporobolomyces odorus*

Thomas Haffner* and Roland Tressl

Institut für Biotechnologie, Fachgebiet Chemisch-Technische Analyse, Technische Universität Berlin, Seestrasse 13, D-13353 Berlin, Germany

Addition of [9,10-$^2H_2$]oleic acid to cultures of the yeast *Sporobolomyces odorus* led to the formation of labeled (*R*)-γ-decanolactone and both enantiomers of (*Z*)-6-γ-dodecenolactone and γ-dodecanolactone. The labeling patterns of these lactones were estimated by a quantitative gas chromatography/mass spectrometry method, suitable for ring-labeled lactones. For the first time, there is strong evidence of oleic acid being a genuine precursor of the important aroma compound (*R*)-γ-decanolactone. On the basis of the presented and former results, a new biosynthetic pathway of this compound is proposed. Starting with a strict enantioselective (*R*)-12-hydroxylation of oleic acid as an initial step, the following β-oxidation led to the lactone. In addition, biosynthetic aspects of the formation of (*Z*)-6-γ-dodecenolactone and γ-dodecanolactone in *S. odorus* are discussed.

Keywords: *γ-Lactones; aroma compound; (R)-γ-decanolactone; [9,10-$^2H_2$]oleic acid; Sporobolomyces odorus; biosynthesis*

INTRODUCTION

γ-Decanolactone (**1**), having a distinct fruity flavor, is a key aroma compound in many fruits. Therefore, **1** is of great interest as a flavor additive used in food and aroma industries (Gatfield et al., 1993). The enantiomeric composition of this chiral compound is characteristic for each kind of fruit. In most cases, (*R*)-**1** is found as the dominating enantiomer (Dufosse et al., 1994). Enantiomeric compositions are used as one criterion in analysis of the origin of foodstuff and flavor additives. Therefore, understanding the basic biosynthetic principles, leading to the different enantiomers of **1** should be of great interest.

Many cultured microorganisms, including *Sporobolomyces odorus*, are able to biosynthesize and accumulate **1** and other γ-lactones (Welsh et al., 1989; Tahara et al., 1975). These organisms can serve as suitable models for investigations concerning the γ-lactone biosynthesis in general. In the past, the precursors of lactones were proven to be hydroxylated fatty acids of C_{18} chain length, which were shortened by β-oxidation steps, followed by lactonization (Albrecht et al., 1992; Cardillo et al., 1989a,b, 1991; Ercoli et al., 1992). The enantiomeric compositions of γ-lactones are different in many microorganisms, too. Under different growing conditions, *S. odorus* produces only (*R*)-**1** (>99%); however, the relative amount of (*R*)-**1** in the fungus *Fusarium poae* varies from 30 to 85% purity (Albrecht and Tressl, 1990; Latrasse et al., 1993). Until now, the natural endogenous C_{18} precursor of **1** could not be detected in γ-lactone-producing microorganisms, but the bioconversion of ricinoleic acid [(*R*)-12-hydroxyoleic acid] into pure (*R*)-**1** has been well-known for more than 30 years and patented for various organisms, including *S. odorus*. This process serves for the industrial production of (*R*)-**1** (Okui et al., 1963; Cheetham et al., 1987; Cardillo et al., 1989a,b; Farbood and Willis, 1983).

The first successful investigations to elucidate the biosynthetic pathway to **1** in *S. odorus* were made by using labeled C_{10} precursors. It could be shown that *S. odorus* converted radioactively labeled [1-^{14}C]decanoic acid into **1**. In contrast, [1-^{14}C]dodecanoic acid did not serve as precursor either for **1** or for (*Z*)-6-γ-dodecenolactone (**2**) (Tressl et al., 1978). In further experiments, the addition of deuterium-labeled, racemic ethyl (*E*)-3,4-epoxydecanoate to cultures of the yeast led to the formation of (*R*)-**1** with a purity of 38% enantiomeric excess (ee). In strawberry tissue homogenate, the same precursor was transformed into (*R*)-**1** with less optical purity than found in the original fruit. In contrast, the racemic (*Z*)-stereoisomer of ethyl 3,4-epoxydecanoate was not a substrate for the biosynthesis of **1** either in *S. odorus* or in strawberry homogenate (Tressl et al., 1988; Albrecht and Tressl, 1990).

Our intentions were to carry out further investigations concerning the unsatisfactory enantiomeric purity of (*R*)-**1** synthesized from the racemic ethyl epoxydecanoate by the yeast. Additionally, it must be assumed that the intermediate compound (*E*)-3,4-epoxydecanoate is formed from (*E*)-3-decenoic acid by the activity of a monooxygenase. Therefore, linoleic acid is supposed to be the natural precursor of (*E*)-3-decenoic acid via β-oxidative degradation. But linoleic acid itself did not serve as a potent precursor of **1**, as results of previous experiments showed (Albrecht, 1991). Only trace amounts of **1** were found labeled after administration of [9,10,12,13-$^2H_4$]linoleic acid to cultures of *S. odorus* (Albrecht et al., 1992). Furthermore, the biosynthesis of ricinoleic acid in seeds of castor bean (*Ricinus Communis L.*) was confirmed to base on oleic acid as fatty acid precursor (Yamada and Stumpf, 1964; James et al., 1965; Galliard and Stumpf, 1966). This led us to the idea to test the ability of [9,10-$^2H_2$]oleic acid to serve as a substrate in the biosynthesis of **1** via ricinoleic acid as an intermediate in *S. odorus*.

In this paper, a new biosynthetic pathway of (*R*)-γ-decanolactone is proposed, on the basis of the gas chromatography−mass spectroscopy (GC−MS) and chiral GC analysis of the metabolites, obtained after administration of [9,10-$^2H_2$]oleic acid to cultures of *S. odorus*. In addition, the biotransformation of [9,10-$^2H_2$]oleic acid into (*Z*)-6-γ-dodecenolactone and γ-dodecanolactone is discussed.

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MATERIALS AND METHODS

[9,10-^{2}H]Oleic Acid. 9-Octadecynoic acid (4.9 g, 17.47 mmol) (98%, Lancaster, U.K.), dissolved in 60 mL of hexane, was hydrogenated under a D_2 atmosphere in the presence of 200 mg of 5% Pd/$BaSO_4$ and 400 μL of freshly destilled quinoline. After the consumption of the required volume of D_2, the quinoline was removed by acidic extraction and the remaining product was washed with brine, dried over $NaSO_4$, and purified on silica gel. A total of 4.46 g (15.67 mmol, 90%) of the product was obtained. The isotopic purity was >97% (^{1}H-NMR).

Mass spectrum as methyl ester (70 eV) *m/z* (intensity, fragment) 298 (1, M^+), 267 (10, $M^+ - 31$), 266 (16), 224 (8), 182 (7), 168 (4), 153 (5), 139 (10), 125 (11), 111 (22), 98 (40), 84 (55), 74 (85), 69 (75), 55 (100), 43 (97). ^{1}H-NMR (500 MHz) (in parts per million): 0.88 (t, 3 H, J = 7.5 Hz, C**H**$_3$), 1.20−1.39 (m, 20 H, C**H**$_2$), 1.63 (quint, 2 H, J = 7.5 Hz, C**H**$_2$CH$_2$-COOH), 1.98−2.04 (m, 4 H, C**H**$_2$CD=CDC**H**$_2$), 2.35 (t, 2 H, J = 7.5 Hz, C**H**$_2$COOH).

Feeding Experiments. *S. odorus* (ATCC 24259) was cultivated in a 1 L Erlenmeyer flask containing 200 mL of medium [45 g/L sucrose, 5 g/L lactose, 3 g/L $MgSO_4 \cdot 7H_2O$, 2.5 g/L $(NH_4)_2SO_4$, 2.5 g/L KH_2PO_4, 2.5 g/L L-alanine, and 0.1 g/L $CaCl_2 \cdot 2H_2O$] at 22 °C and 80 rpm (Albrecht, 1991). After 96 h of incubation, 50 mg (176 μmol) of [9,10-^{2}H$_2$]oleic acid dissolved in 150 μL of ethanol was added. At different time points, 10 mL aliquots were removed from the culture broth and extracted with diethyl ether (experiment I). Following treatment with diazomethane, the extracts were analyzed by GC and GC/MS. In a second experiment (II) and under the same cultivation conditions, 200 mg (704 μmol) of [9,10-^{2}H$_2$]-oleic acid, dissolved in 300 μL of ethanol, was added to 200 mL of sterile medium before inoculation. After 220 h, the whole broth was extracted with diethyl ether, and after concentration to 0.2 mL, the extract was separated on a LSC column (6.5 g of silica gel) into five 40 mL fractions [I, pentane, II, pentane/CH_2Cl_2 (9:1); III, pentane/CH_2Cl_2 (2:1); IV, pentane/diethyl ether (9:1); V, diethyl ether]. Fraction IV contained all of the lactones (Albrecht, 1991).

Gas Chromatography (GC)/Mass Spectrometry (MS)/NMR. If necessary, the extracts were saturated with diazomethane, and following concentration to 0.2 mL, 1 μL was subjected to GC analysis on a capillary column (DB-Wax 60 M, 60 m × 0.32 mm inside diameter, J&W, Folsom, CA) and a chiral column (Lipodex E, 50 m, Machery & Nagel, Germany), both coupled to a FID detector or for the DB wax column additionally connected to the mass spectrometer. The mass spectra were recorded on a Varian CH 5-DF (Varian, Germany) double-focusing mass spectrometer at 70 eV ionization energy, combined with a MS data system DP 10 (AMD, Germany). ^{1}H-NMR spectra were recorded on a Bruker AMX 500 NMR instrument with $CDCl_3$ as solvent.

RESULTS

[9,10-^{2}H$_2$]Oleic acid was administered to *S. odorus* cultures. At different time points, aliquots of the culture broth were taken and the formation of volatiles was monitored by GC/MS analysis. In Figure 1, mass spectra of **1**, (*Z*)-6-γ-dodecenolactone (**2**), and γ-dodecanolatone (**3**) are presented, 147 h after addition of 250 ppm labeled substrate, representing the three labeled γ-lactones formed during the cultivation. The fragment ion *m/z* 85 (tetrahydropyranonyl cation = base peak) is the most important in the mass spectral analysis of γ-lactones with saturated ring moieties. In addition, the prominent ions *m/z* 86 and 87 were detected, thus demonstrating the content of the deuterium-labeled lactones, containing one or two deuterium atoms in the ring part. In our experiments, isotopomere lactones showed a partial resolution on capillary GC colums. Consequently, an isolated MS scan was not representative for the quantification of the label. More reliable results were obtained by integrating the fragment ions

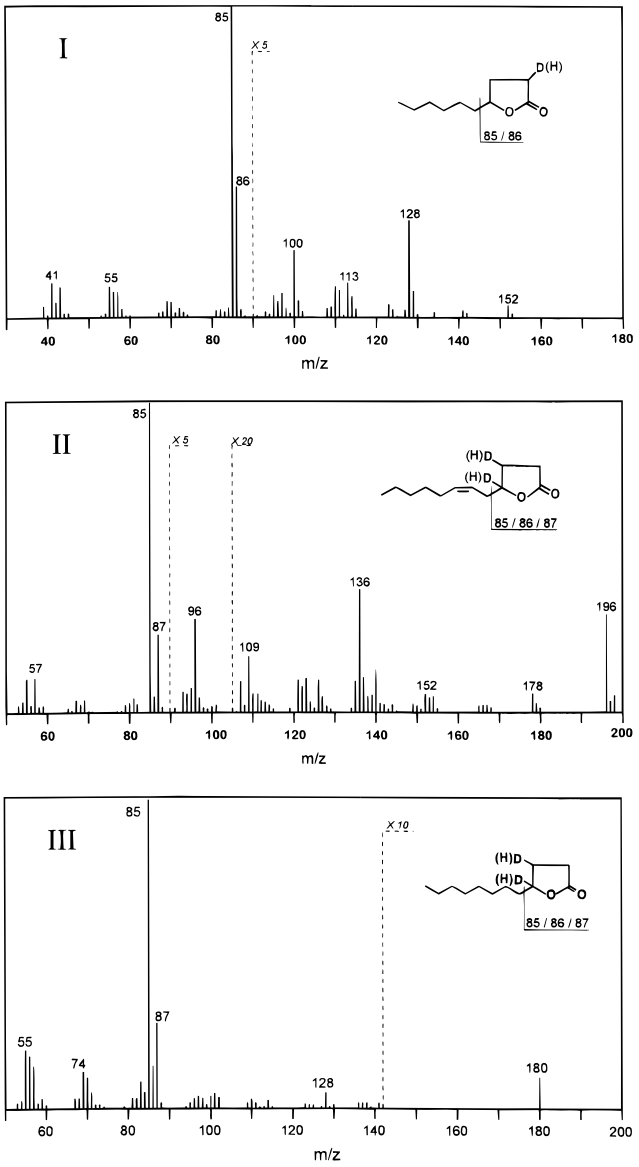


Figure 1. EI-mass spectra of partially labeled γ-decanolactone (I), (*Z*)-6-γ-dodecenolide (II), and γ-dodecanolactone (III) found in cultures of *S. odorus* 147 h after the addition of 250 ppm [9,10-^{2}H$_2$]oleic acid to growing cells of the yeast (conditions, experiment I).

m/z 85, 86, and 87 over all MS scans representing the corresponding lactone. Therefore, the single ion monitoring mode of the MS software was used. Before calculation of the labeling pattern, the naturally occurring isotopic pattern of the mass fragment *m/z* 85 was taken into account.

In Figures 2 and 3, the time dependent formation of labeled and unlabeled **1** and **2** after addition of 250 ppm [9,10-^{2}H$_2$]oleic acid is shown (experiment I). In contrast to the lactones **1** and **2**, labeled **3** was detected for the first time 96 h after the substrate addition. Hence, a quantification of **3** compared to **1** and **2** was not feasible. Until 147 h, the amounts of labeled **1** and **2** increased. At the same time, no labeled oleic acid could be detected anymore, but linoleic acid found to be labeled in a relative amount of about 10−20% (Figure 4). After this time, only 5−25 ppm oleic acid, biosynthesized endogenously, was found, which served as substrate for the unlabeled lactones furthermore. Overall, less than 3% of the precursor was transformed into the three γ-lactones, with the remaining amount suggested to be metabolized via β-oxidation or incorporated into the triglycerides and phospholipids, respectively.

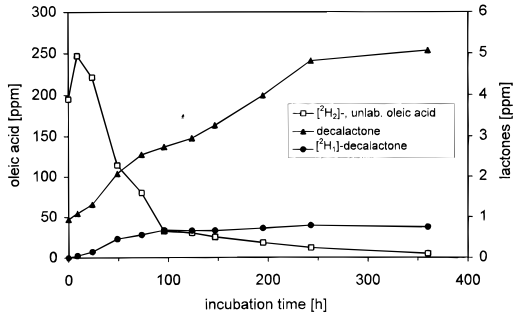


Figure 2. Concentration of [2-$^2H_1$]-γ-decanolactone and γ-decanolactone during the fermentation after addition of 250 ppm [9,10-$^2H_2$]oleic acid to growing cells of *S. odorus*.

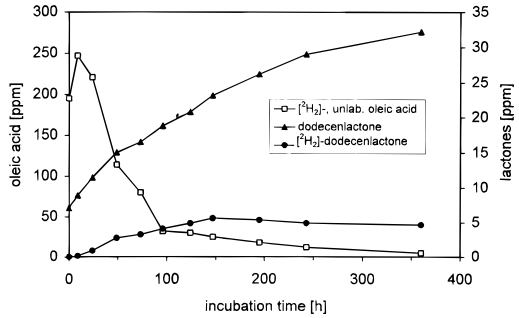


Figure 3. Concentration of [3,4-$^2H_2$]-(*Z*)-6-γ-dodecenolactone and unlabeled (*Z*)-6-γ-dodecenolactone during the fermentation after addition of 250 ppm [9,10-$^2H_2$]oleic acid to growing cells of *S. odorus*.

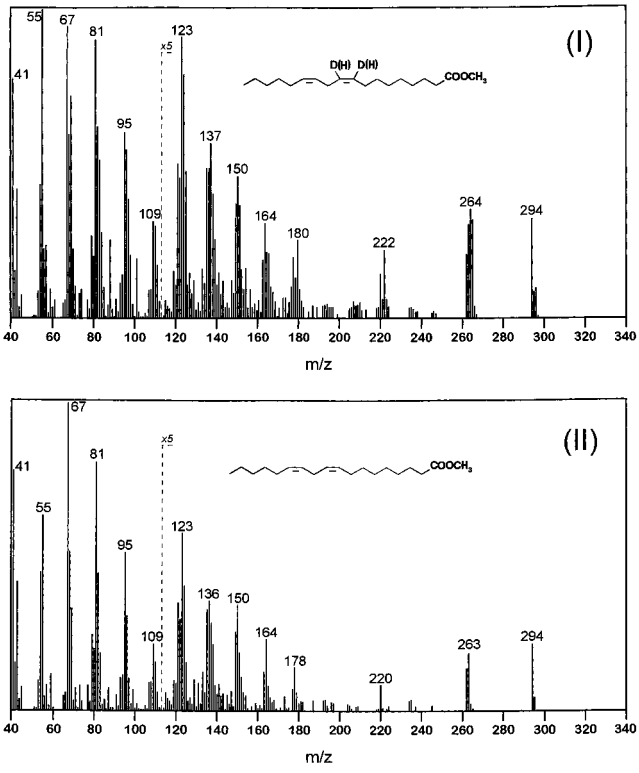


Figure 4. (I) EI-mass spectrum of [9,10-$^2H_2$]methyl linoleate mixed with unlabeled methyl linoleate found in cultures of *S. odorus* 147 h after the addition of 250 ppm [9,10-$^2H_2$]oleic acid to growing cells of the yeast (conditions, experiment I) and (II) EI-mass spectrum of unlabeled methyl linoleate as reference.

Under the above conditions (experiment I) and compared to original reference cultures, the addition of 250 ppm [9,10-$^2H_2$]oleic acid influenced neither the formation rate nor the amount of accumulated lactones. The ratio of **1** to **2** was found to be 1 to 6−7 over the whole fermentation time. In reference cultures, the ratio was constant at 1 to 3−4. In contrast, addition of 1000 ppm [9,10-$^2H_2$]oleic acid to the medium before inoculation (experiment II) caused an opposite effect. The relative ratio of **1** to **2** was 1 to 1.2, 220 h after fermentation started. At this time, the concentration of **1** was 10.8 ppm and that of **2** was 12.7 ppm (Table 1).

Table 1. Concentration and Relative Amounts of 1- and 2-Fold-Labeled γ-Decanolactone, (*Z*)-6-γ-Dodecenolactone, and γ-Dodecanolactone Found after Addition of 250 and 1000 ppm [9,10-$^2H_2$]Oleic Acid to Cultures of *S. odorus*[a]

	[9,10-$^2H_2$]oleic acid							
	exp I 250 ppm (147 h)				exp II 1000 ppm (220 h)			
lactone	concn (ppm)	[$^2H_1$] (%)	[$^2H_2$] (%)	total label (%)	concn (ppm)	[$^2H_1$] (%)	[$^2H_2$] (%)	total label (%)
1	4.1	16.8	<0.5	17.3	10.8	47.5	2.8	50.3
2	28.7	1.6	19.5	21.1	12.7	2.8	26.8	29.6
3	<0.01	nd	nd	nd	1.1	15.8	33.4	49.2

[a] Experiments I and II, see Materials and Methods. nd is not determined.

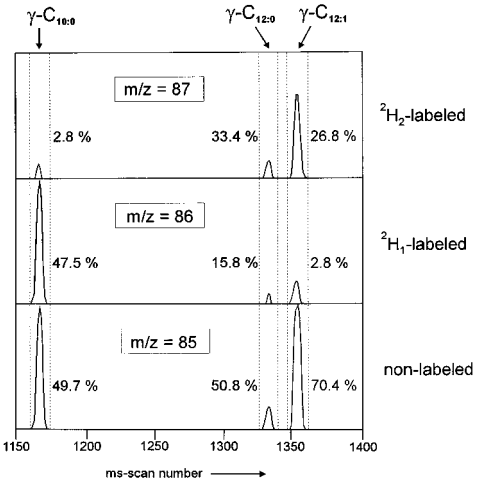


Figure 5. Capillary gas chromatogram (reconstructed ion detection) of the lactone fraction (conditions, experiment II) operating in the single ion monitoring mode and quantification of the mass fragments *m/z* 85, 86, and 87 [DB-Wax 60 M, 60 m × 0.32 mm inside diameter, 70 °C (4 min isothermal) to 220 °C at 4 °C/min].

The enhanced metabolization of labeled oleic acid to **1** can also be demonstrated by the relative content of the labeled lactones (Figure 5). The whole labeling content of **1** was 50.3%; only a small amount of 2.8% was labeled 2-fold. Lactone **2** was found labeled in a relative quantity of 29.6% with an 2.8% amount of 1-fold labeling. The biosynthesis of lactone **3** increased, too. After 220 h, a concentration of 1.1 ppm was detected. A total of 49.2% of **3** was found labeled, but different from **2**, 15.8% of **3** was only labeled once and 33.4% contained a second deuterium atom.

In Figure 6, the chiral analysis of the lactone fraction 147 h after the addition of [9,10-$^2H_2$]oleic acid is shown. The enantiomeric excess (ee) of the (*R*)-enantiomers of **1** and **2** was found to be >99 and 84%, respectively. These values are identical to those found in original cultures of *S. odorus* before (Albrecht, 1991; Albrecht et al., 1992). On the basis of these results, an isotope effect on the enzymes involved in the formation of the chirality center, caused by the deuterium label, can be excluded. For the first time, the enantiomeric composition of γ-dodecanolactone in *S. odorus* could be analyzed.

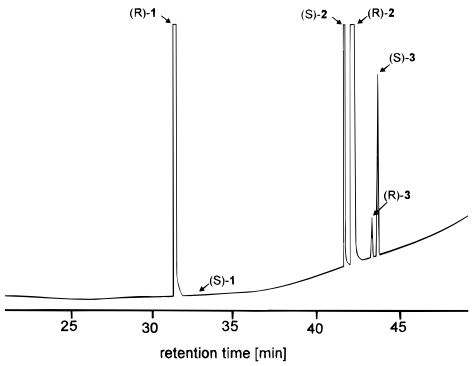


Figure 6. Chiral capillary gas chromatogram of the lactone fraction isolated from growing cultures of *S. odorus* 147 h after addition of [9,10-$^2H_2$]oleic acid [conditions, experiment I; Lipodex E, 50 m × 0.25 mm inside diameter, 150 °C (20 min isothermal) to 220 °C at 2 °C/min; carrier gas, He].

In contrast to the excess of the (*R*)-enantiomers of **1** and **2**, lactone **3** contained the (*S*)-enantiomer in 59% ee.

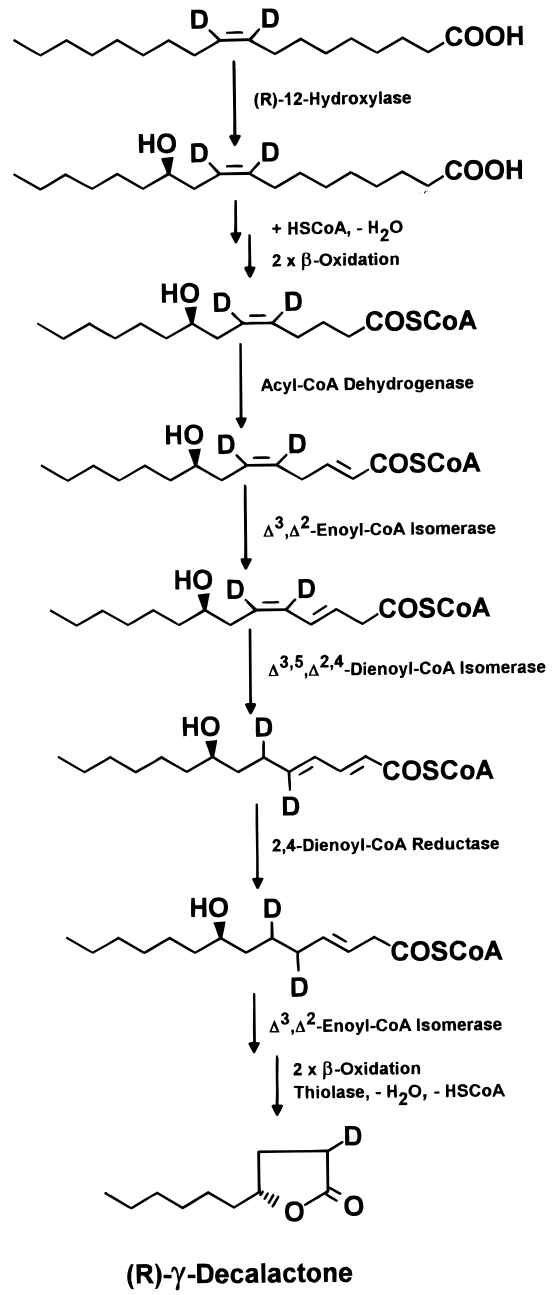
DISCUSSION

For the first time, there is strong evidence of oleic acid being a genuine precursor of the important aroma compounds (*R*)-γ-decanolactone and γ-dodecanolactone, (*Z*)-6-γ-dodecenolactone in a microorganism. Along with linoleic acid, now oleic acid must be seen as a potent natural substrate in the biosynthesis of γ-lactones in the yeast *S. odorus*.

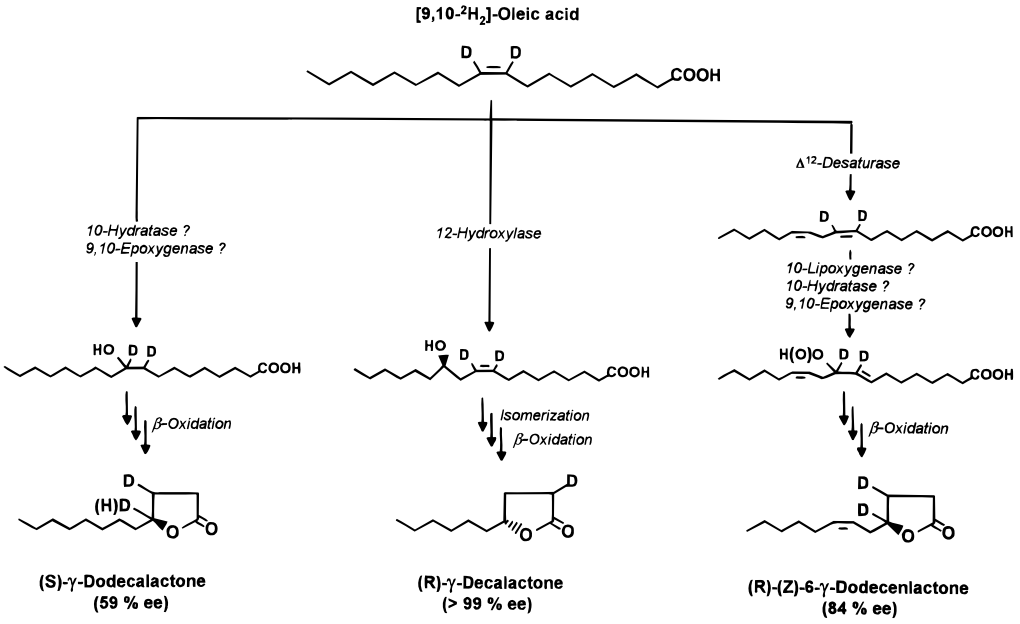
Concerning the biosynthesis of γ-decanolactone, we suggest a strictly stereospecific, enzymatic introduction of the hydroxyl group to oleic acid at carbon-12 by the action of a hydroxylase. This kind of microbial monooxygenase activity, introducing a (*R*)-12-hydroxy group to oleic acid, was characterized first in *Bacillus pumilis*. Cultures of this organism convert 72% of added oleic acid to ricinoleic acid after 96 h without further metabolic degradation (Kenji and Kido, 1989). A second parallel can be drawn to the biosynthesis of ricinoleic acid in castor bean seeds, where oleic acid had been proven to be the precursor of the enantiomerical pure ricinoleic acid [(*R*)-12-hydroxyoleic acid] (Yamada and Stumpf, 1964; James et al., 1965; Galliard and Stumpf, 1966). Our idea is supported by the ability of *S. odorus* to metabolize ricinoleic acid to (*R*)-γ-decanolactone rapidly in high yield (Albrecht et al., 1992). Scheme 1 summarizes the proposed enzymatic steps from [9,10-$^2H_2$]oleic acid to [2-$^2H_1$]-(*R*)-**1** on the basis of known biochemical pathways of fatty acid degradation. (*R*)-12-Hydroxyoleic acid is activated by the conversion to its CoA ester and shortened by two β-oxidation cycles leading to (*R*)-8-hydroxy-(*Z*)-5-tetradecenoyl-CoA. This acid CoA possesses a (*Z*)-configured Δ^5-double bond, which cannot be metabolized without isomerizing the double bond to position trans-2 first as Smeland et al. (1992) and Chen et al. (1995) demonstrated. The next step in this reaction cascade will be the introduction of an additional trans-2 double bond to the molecule by the action of an acyl-CoA dehydrogenase, leading to (*R*)-8-hydroxy-(*E,Z*)-2,5-tetradecadienoyl-CoA. Further enzymatic transformation steps, including the key activity of a $\Delta^{3,5},\Delta^{2,4}$-dienoyl-CoA isomerase, led finally to (*R*)-8-hydroxy-(*E*)-2-tetradecenoyl-CoA which serves as substrate for two further β-oxidation steps, resulting in (*R*)-4-hydroxydecanoyl-CoA, which lactonizes to **1** after hydrolysis of the CoA ester. To consolidate this postulated pathway in *S. odorus*, more detailed investigation of this enzymatic system will be necessary, including isolation and characterization of intermediates. Especially the occurrence of oleic acid, either as free acid or esterified to triglycerides or phospholipids, as well as the elucidation of the key step, the enzymatic 12-hydroxylation remains very interesting. In microsomes of castor bean seeds, there was evidence of a cytochrome b_5 acting as the electron donor in this reaction. Cytochrome P-450 was not involved here. Additionally, Bafor et al. (1991) found that only oleic acid esterified to phosphatidylcholine can serve as substrate for the hydroxylation reaction. Neither the free acid nor oleoyl-CoA is a suitable substrate for this reaction catalyzed by microsomal enzymes in castor bean.

Former results concerning the lactone formation in the yeast showed that [9,10,12,13-$^2H_4$]linoleic acid was not converted into **1** significantly but served as an excellent substrate for the biosynthesis of **2** and few other γ- and δ-lactones (Albrecht, 1991; Albrecht et al., 1992). These results and the findings that ethyl (*E*)-3,4-epoxydecanoate and its corresponding acid, which are supposed to originate from linoleic acid by β-oxida-

Scheme 1. Proposed Transformation of [9,10-$^2H_2$]-Oleic Acid to [2-$^2H_1$]-(*R*)-γ-Decanolactone by Whole Cells of *S. odorus*



Scheme 2. Postulated Metabolic Steps of [9,10-$^2H_2$]Oleic Acid to γ-Lactones in *S. odorus*



tion and epoxidation, are precursors of **1** are inconsistent. More experimental data are needed to solve the discrepancy.

The presented results demonstrate the ability of *S. odorus* to synthesize two additional lactones (**2** and **3**) from oleic acid as a natural precursor. Taking into account the successful transformation of linoleic acid into **2**, the activity of a Δ^{12}-desaturase as the initial enzymatic step in the metabolic pathway of oleic acid leading to **2** must be assumed. There is evidence for this step, because 147 h after the addition of [9,10-$^2H_2$]-oleic acid (experiment I) about 15−20% of the linoleic acid was found to be deuterium-labeled (Figure 4) (quantification of the label in linoleic acid could not be as exact as for the lactones, because of the loss of deuterium during fragmentation of the molecule in the mass spectrometer). Other linoleic acid-derived lactones could not be detected in labeled form, because their concentration in the culture broth was too low under the chosen experimental conditions (δ-decanolactone) or the label was separated during the metabolic degradation of the linoleic acid derivatives (γ-nonanolactone).

The enzymatic introduction of the required 10-hydroxyl group to linoleic acid as the initial step in the presented pathway to **2** still remains unclear. In view of these results, there was neither direct evidence for the activity of a 10-lipoxygenase, comparable to mushrooms (Wurzenberger and Grosch, 1984), nor the action of a 10-hydratase, or alternatively an activity of a 9,10-epoxygenase/hydrolase. In contrast to our results where (*R*)-**2** was the dominant product (application of the Cahn−Ingold−Prelog rules causes a formal inversion of the stereocenter), the stereospecificity of microbial 10-hydration of the Δ^9-double bond in linoleic acid always led to an excess of the (*R*)-10-hydroxy enantiomer, which would lead to (*S*)-**2** after metabolic degradation (Hou, 1994). Compared with that, all known lipoxygenases catalyze in a (*S*)-stereospecific mode, but there is no specific information about the stereochemical course of 10-lipoxygenases in microorganisms until now. Therefore, more detailed investigation of this initial enzymatic step is necessary to identify the involved enzyme(s) in *S. odorus*.

For the first time, γ-dodecanolactone, containing an excess of the (*S*)-enantiomer, was identified as a metabolite of oleic acid in *S. odorus*. Obviously, the biosynthesis of **3** requires the introduction of a hydroxyl function to oleic acid at carbon-10. Microbial hydrations of oleic acid to 10-hydroxystearic acid performed by bacteria and yeasts are well-known and occur with different enantioselectivities, but always with an excess of the (*R*)-isomer (Wallen et al., 1962; El-Sharkawy et al., 1992; Yang et al., 1993). The enantioselectivity of the hydration is often modified by a following oxidation/reduction step of the 10-hydroxystearic acid catalyzed by dehydrogenases (W. Albrecht and R. Tressl, unpublished results). However, the occurrence of the two different labeled isotopomers of **3** requires an explanation. For [$^2H_1$]-**3** we assume an enzymatic inversion of the stereocenter at carbon-10, including a loss of one deuterium atom. To confirm this hypothesis, a mass spectral analysis of the two separated enantiomers of **3** would be helpful. In our investigations, this analytical procedure failed because of the very low concentration of this compound produced by the yeast. Further detailed investigations are in progress.

The activities of the involved enzymes can be modified by exposing the organism to different concentrations of oleic acid. Addition of 250 ppm oleic acid to 96 h old cultures of the yeast enhanced the production of **2** compared to that of **1**, via the desaturase pathway. Administration of 1000 ppm oleic acid to the culture broth before inoculation favors the formation of **1** and led to a decreased production of **2**. Under these conditions, **3** was synthesized in an about 100-fold concentration, compared to reference cultures where it could be detected only in trace amounts (<10 ppb). This finding can be explained by a positive induction of the proposed hydroxylation activity leading to (*R*)-12-hydroxyoleic acid.

Our results, summarized in proposed pathways (Scheme 2), indicate that the biotransformation of oleic acid to γ-lactones in *S. odorus* is complex and seems to be based on at least three different metabolic pathways. For (*R*)-γ-decanolactone, a strict (*R*)-12-enantioselective hydroxylation of oleic acid, catalyzed by a hydroxylase, should be the first enzymatic step, followed by four β-oxidation cycles and dehydration, leading to the lactone. Parallel to this pathway, a Δ^{12}-desaturase activity leading to linoleic acid was confirmed by the

characterization of the labeled double-unsaturated fatty acid. The alternative first steps in the subsequent metabolic degradation of linoleic acid and oleic acid still remain unclear. They could be catalyzed by a 10-lipoxygenase and/or a 10-hydratase or 9,10-epoxygenase for (*Z*)-6-γ-dodecanolactone or by the last two enzymes for the saturated γ-dodecanolactone. The identification of these enyzmatic activities in *S. odorus* is the subject of our current research and needs further investigation, especially in the isolation of significant intermediates and in the characterization of the involved enzymes in cell free systems.

ACKNOWLEDGMENT

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