174. Biosynthesis of δ-Jasmin Lactone (= (*Z*)-Dec-7-eno-5-lactone) and (*Z*,*Z*)-Dodeca-6,9-dieno-4-lactone in the Yeast *Sporobolomyces odorus*

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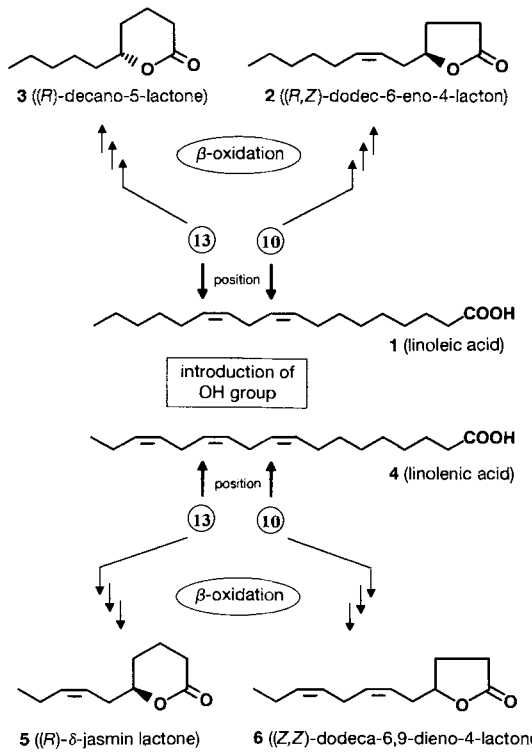
(all-*Z*)-(9,10,12,13,15,16-$^2H_6$)Octadeca-9,12,15-trienoic acid (= α-linolenic acid; D_6-**4**) was synthesized to investigate the biochemical formation of linolenic-acid-derived aroma compounds in cultures of the yeast *Sporobolomyces odorus*, using an established gas chromatographic/mass spectrometric (GC/MS) method. Three compounds were identified as labeled: (*Z*)-dec-7-eno-5-lactone (δ-jasmin lactone), (*Z*,*Z*)-dodeca-6,9-dieno-4-lactone, and (2*E*,4*Z*)-hepta-2,4-dienoic acid. Both lactones were biosynthesized mostly under conservation of the initial configuration from their corresponding oxygenated linolenic-acid intermediates. The application of (13*S*,9*Z*,11*E*,15*Z*)-13-hydroxy(9,10,12,13,15,16-$^2H_6$)octadeca-9,11,15-trienoic acid (D_6-**7**) as a OH-functionalized precursor of δ-jasmin lactone allowed to gain insight into the stereochemical course of the biosynthesis to both enantiomers of this lactone. In this experiment, 88.3% of the metabolized labeled precursor was transformed under retention of the original configuration of the (*R*)-enantiomer. This investigation is also a contribution to a better understanding of the C=C bond isomerization steps which took place during the β-oxidative degradation of the substrate.

Introduction. – Chiral γ- and δ-lactones make significant contributions to the sensory properties of many foods. In contrast to their important role in flavor research and industry, the biogenetic pathways to the aroma compounds are often unknown. The chirospecific analysis of γ- and δ-lactones (by means of enantiomer separation by gas chromatography (GC) on chiral capillary columns) is an effective tool to prove the authenticity of natural flavorings in food. Therefore, the knowledge of the biosynthetic pathways to lactone enantiomers is of importance. Recently, the biosynthesis of dodecano-4-lactone in peaches, strawberries, and some other fruits was investigated by using suitable deuterated fatty acid precursors [1]. Other experiments performed with strawberries demonstrated that (*E*)-dec-3-enoic acid can serve as a substrate in the biosynthesis of decano-4-actone [2] [3]. However, vegetable systems have some disadvantages, such as the dependency of the corresponding biochemical activity from the season and the degree of fruit ripeness. As appropriate models, lactone-producing microorganisms were successfully used to overcome these restrictions. Aspects of the microbial pathways to decano-5-lactone and some γ-lactones were studied by using labeled fatty acids or oxygenated fatty acids as precursors [3–10]. The dominant pathway to (*R*)-decano-4-lactone in the yeast *Sporobolomyces odorus* was characterized as a (*R*)-12-hydroxylation of oleic acid (= (*Z*)-octadec-9-enoic acid) followed by β-oxidation. In this study, oleic acid was also transformed into linoleic acid (= (*Z*,*Z*)-octadeca-9,12-dienoic acid; **1**), which subsequently was degraded to (*Z*)-dodec-6-eno-4-lactone (**2**), possessing an enantiomer ratio (*R*)/(*S*) of 92:8 (see *Scheme 1*). The biosynthesis of (*R*)-decano-5-lactone (**3**; (*R*) > 99%) in *S. odorus* is initiated by a lipoxygenation at C(13) of linoleic acid (**1**). It could be shown

that administered (13*S*)-13-hydro(pero)xyoctadeca-9,11-dienoic acid was converted to (*R*)-decano-5-lactone (**3**) under total inversion of the initial configuration (*Scheme 1*) [4–6].

Scheme 1. *Biochemical Formation of Different Lactones from 10- and 13-Hydroxylated Linoleic and Linolenic Acid in* S. odorus



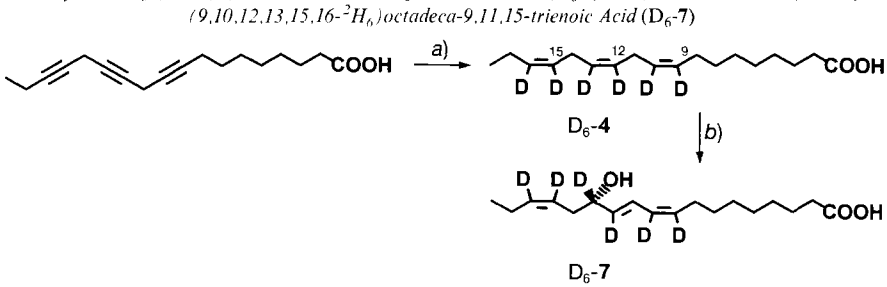
δ-Jasmin lactone (= (*Z*)-dec-7-eno-5-lactone; see **5** for (*R*)-enantiomer) possesses a fruity, sweet floral aroma and occurs in a variety of different foods, *e.g.* cheddar cheese, tea, peaches, mangoes, and in flower extracts from *Jasminum*, *Gardenium*, *Tuberose*, and *Mimosa* species as well [11–13]. Its chemical structure, containing a (*Z*)-configurated C=C bond at the ω − 3 position, was confirmed first by synthesis of its racemic form in 1962 by *Demole et al.* [14]. Both enantiomers occur in nature. Their absolute configuration was assigned by *Blaser et al.* by means of a chiral synthesis [15]. Extracts of *Jasminum* flowers contain the lactone in the pure (*R*)-configuration, whereas in *Tuberose* species, only the (*S*)-enantiomer is biosynthesized. However, in most plants, this lactone is produced in distinct enantiomer ratios. As a microbial product, δ-jasmin lactone was identified for the first time in cultures of the yeast *S. odorus* [16]. The enantiomer composition of this compound extracted from two different strains of *S. odorus* was determined by means of a GC analysis [17] [18].

In this paper, we would like to present the results of our investigations concerning the influence of a C=C bond at the ω − 3 position in the course of the formation of linolenic-

acid-derived lactones in *S. odorus*. Therefore, ($^2H_6$)linolenic acid (= α-($^2H_6$)linolenic acid) D_6-**4** and its (*S*)-13-hydroxy($^2H_6$) derivative D_6-**7** were synthesized and used as precursors to study the stereochemical course of the biosynthesis of δ-jasmin lactone and dodeca-6,9-dieno-4-lactone.

Results. – To investigate the biosyntheses of linolenic-acid-derived flavor compounds, (*Z*,*Z*,*Z*)-(9,10,12,13,15,16-$^2H_6$)linolenic acid (D_6-**4**) was synthesized using a convergent concept, which based on well known alkyne chemistry [19] (*Scheme 2*). Acid D_6-**4** which carried the D-atoms at the olefinic C-atoms has decisive advantages over an acid with a label in the saturated parts of the molecule. In biochemical processes in which polyunsaturated fatty acids are involved, the penta-1,4-dienyl structures are often the site of the interesting enzymatic activities (*e.g.* by lipoxygenases, dehydrogenases, isomerases). Conservation or loss of the label at these positions often refer to pathways and enzyme mechanisms in the metabolism of these fatty acids. In our synthetical approach to D_6-**4**, starting from 3-(tetrahydro-2*H*-pyran-2-yloxy)prop-1-yne, we used some more effective steps for the synthesis of several intermediates. This improved the overall yield of D_6-**4** to more than 12% ([19]: 0.5%). The (13*S*)-13-hydroxy derivative D_6-**7** was then obtained by lipoxygenation followed by $NaBH_4$ reduction (*Scheme 2*).

Scheme 2. *Synthesis of (all-Z)-(9,10,12,13,15,16-$^2H_6$)Linolenic Acid* (D_6-**4**) *and (13S,9Z,11E,15Z)-13-Hydroxy-(9,10,12,13,15,16-$^2H_6$)octadeca-9,11,15-trienoic Acid* (D_6-**7**)



a) D_2, *Lindlar*'s catalyst, quinoline. *b*) 1. Soybean lipoxygenase, pH 9.0, 2. $NaBH_4$.

The quantitative analyses of the labeling patterns at the six olefinic C-atoms of D_6-**4** and of derivative D_6-**7** were performed by ^{1}H-NMR spectroscopy, with unlabeled linolenic acid (**4**) and (13*S*,9*Z*,11*E*,15*Z*)-13-hydroxyoctadeca-9,11,15-trienoic acid (**7**) as reference compounds (D_6-**4**: C(9) (97.2%), C(10) (96.3%), C(12) (95.8%), C(13) (97.3%), C(15) (96.2%), C(16) (96.8%); D_6-**7**: C(9) (97.9%), C(10) (97.3%), C(12) (96.4%), C(13) (98.0%), C(15) (96.4%), C(16) (96.2%)). Both analyses are highly correlated and showed that the lipoxygenation of D_6-**4** and the following $NaBH_4$ reduction did not cause a D-depletion in the molecule.

After addition of 53 mg of D_6-**4** to a 200-ml shake-flask culture (= 265 ppm) of *S. odorus*, δ-jasmin lactone (*Kovats* index$_{DB\,wax}$ (K_{iDBwax}) = 2225), (*Z*,*Z*)-dodeca-6,9-dieno-4-lactone (K_{iDBwax} = 2443), and (2*E*,4*Z*)-hepta-2,4-dienoic acid (K_{iDBwax} = 1467) could be identified as labeled by mass spectrometry (*i.e.*, [2,4,5,7,8-D]-**5**, [3,4,6,7,9,10-D]-**6**, and [2,4,5-D]-**8**), their *Kovats* indices, and by comparison with unlabeled reference compounds. After addition to the cultures, D_6-**4** was totally consumed by the yeast within 24 h. After this time, only unlabeled linolenic acid (**4**) was detected in extracts of the culture broths in a concentration range between 5 and 8 ppm. In total, less than 1% of the

added fatty acid was transformed into one of the three labeled compounds. The major part of D_6-**4** is assumed to be metabolized to acetate or built into triglycerides or phospholipids, non-volatile products which are not detectable by the applied analytical methods. Nine hours after administration of the precursor, all three metabolites occurred labeled for the first time. Compared to a non-fed reference culture, only the concentration of dodeca-6,9-dieno-4-lactone (0.2–1.4 ppm) increased after addition of D_6-**4**. The two other metabolic products were found in comparable concentrations to non-fed cultures (0.1–0.8 ppm). During the biotransformation, the three labeled compounds were established to be mixtures of labeled and unlabeled compounds. The reason for this finding was the constant level of endogenous linolenic acid (**4**), produced by the microorganism from the unlabeled carbon source *via* its *de novo* fatty acid biosynthetic pathway. The mass spectra of mixtures of labeled/unlabeled compounds and the pure unlabeled reference compounds are presented in *Fig. 1*.

The label, which was bound to the ring moiety of both lactones, was quantified by a GC/MS method applied to γ-lactones before [4]. One of the major advantages of this method is the possibility to use labeled precursors of 'natural' chain lengths in presence of the unlabeled endogenous precursor. This can be very important in the analysis of biosynthetic pathways to chiral compounds. Fatty-acid derivatives possessing an 'unnatural' chain length, as recently used for investigations of the biosynthesis of dodecano-4-lactone in fruits, influenced the stereochemistry of the processes involving enzymes [1].

To verify our analytical method as suitable for investigations of δ-lactones, we analyzed (*R*)-(2,3-$^2H_2$)decano-5-lactone (D_2-**3**), which was synthesized from massoia lactone (= (*R*)-dec-2-eno-5-lactone) by PtO_2-catalyzed reduction with D_2. Parallel GC/MS and ^{1}H-NMR analysis of the labeled und unlabeled δ-lactones D-**3** and **3**, respectively, were performed. We calculated an average deuterium content of 1.94 D-atoms for position C(2) and C(3) of D_2-**3** from the ^{1}H-NMR spectra. Using the GC/MS method, we determined 95.2% of double-, 4.1% of single-, and 0.7% of non-labeled lactone. These corresponding results confirmed the value of this EI-MS technique for the quantitative analysis of the label located in the ring part of deuterated δ-lactones.

Between 80 and 120 h after administration of D_6-**4** to cultures of the growing yeast, the quantity of the labeled isotopomers reached their optimum. Therefore, 96-h old culture extracts were used to analyze the D-content of the products by means of the achiral GC/MS method. For the labeled δ-jasmin lactone, the isotopomers D_5- (36.3%), D_4- (25.7%), and D_3-**5** (9.9%) were found (*Table 1*). We also detected non-labeled lactone **5** (28.1%). Thus, in the course of the biotransformation of D_6-**4** to δ-jasmin lactone, the major part of the lactone was synthesized without loss of D-atoms, but also up to 2 D-atoms were eliminated and exchanged by H-atoms at the positions C(10), C(12), or C(13) (*i.e.*, C(2), C(4), or C(5) of the resulting lactone) of the fatty acid precursor.

For the labeled dodeca-6,9-dieno-4-lactone from the same extract (96 h incubation), 62.4% of D_6-**6** (= 84.2% of the labeled part) and 11.7% of D_5-**6** (= 15.8% of the labeled part) were found, whereas the remaining 25.9% of this lactone originated from the unlabeled endogenous linolenic acid (*Table 2*). Thus, during the biosynthesis of this lactone, most of the original label was conserved.

Further investigations to determine the labeling patterns of the separated enantiomers of both lactones, as a result of the linolenic acid experiment, failed because of their low concentration in the complex compound mixture found in the culture extracts.

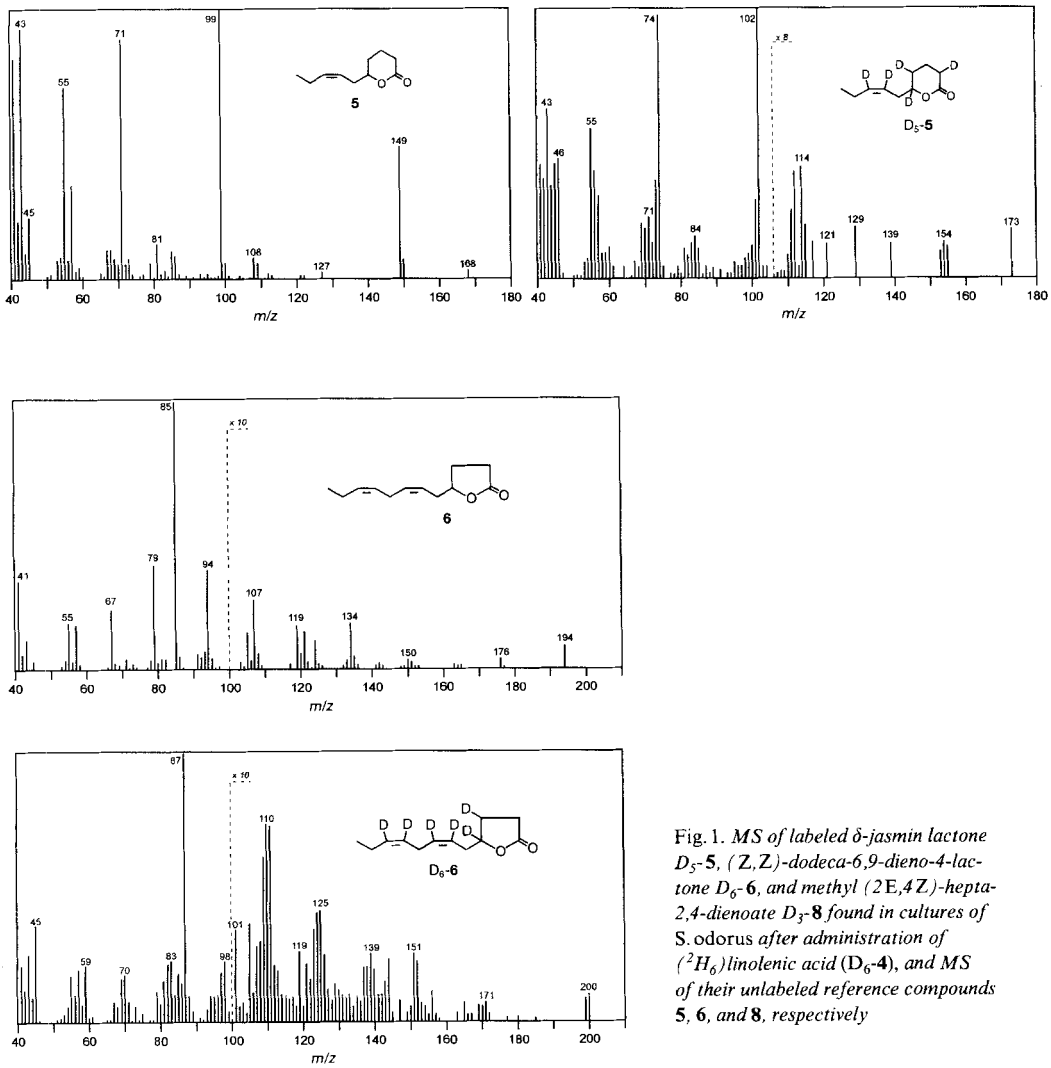


Fig. 1. *MS of labeled δ-jasmin lactone D_5-***5**, *(Z,Z)-dodeca-6,9-dieno-4-lactone D_6-***6**, *and methyl (2E,4Z)-hepta-2,4-dienoate D_3-***8** *found in cultures of* S. odorus *after administration of ($^2H_6$)linolenic acid* (D_6-**4**), *and MS of their unlabeled reference compounds* **5**, **6**, *and* **8**, *respectively*

Table 1. *Relative Distribution of the Isotopomers of δ-Jasmin Lactone [2,4,5,7,8-D]-5 in Cultures of* S. odorus *Formed 96 h after Addition of 53 mg of ($^2H_6$)Linolenic Acid* (D_6-**4**) *or Formed 102 h after Addition of 80 mg of (13S,9Z,11E,15Z)-13-Hydroxy($^2H_6$)octadeca-9,11,15-trienoic Acid* (D_6-**7**) (capillary column: *DB* wax)

	Representing mass fragment	Amount [%] from substrate	
		D_6-**4**	D_6-**7**
Unlabeled δ-jasmin lactone (**5**)	99	28.1	32.8
δ-($^2H_3$)Jasmin lactone (D_3-**5**)	100	9.9	8.1
δ-($^2H_4$)Jasmin lactone (D_4-**5**)	101	25.7	23.8
δ-($^2H_5$)Jasmin lactone (D_5-**5**)	102	36.3	35.3

Table 2. *Relative Distribution of the Isotopomers of (Z,Z)-dodeca-6,9-dieno-4-lactone [3,4,6,7,9,10-D]-6 Formed 96 h after Addition of 53 mg of ($^2H_6$)Linolenic Acid* (D_6-**4**) *to Cultures of* S. odorus (capillary column: *DB wax*)

	Representing mass fragment	Amount [%] from substrate D_6-**4**
Unlabeled dodeca-6,9-dieno-4-lactone (**6**)	85	25.9
($^2H_5$)Dodeca-6,9-dieno-4-lactone (D_5-**6**)	86	11.7
($^2H_6$)Dodeca-6,9-dieno-4-lactone (D_6-**6**)	87	62.4

To prove a lipoxygenation as the initial step in the biosynthesis of δ-jasmin lactone, (13*S*,9*Z*,11*E*,15*Z*)-13-hydroxy(9,10,12,13,15,16-$^2H_6$)octadeca-9,11,15-trienoic acid (D_6-**7**) was applied to cultures of the yeast as a potential precursor. After biotransformation of 80 mg of D_6-**7**, using two 200-ml shake-flask cultures of *S. odorus* over a period of 102 h, the lactone was purified by prep. GC. Less than 10% of D_6-**7** was transformed into the lactone. Analytical investigations were performed by achiral and chiral GC. As the achiral analysis revealed, the relative distribution of the isotopomeric jasmin lactones correlated with those found in the experiments using D_6-**4** as precursor (*Table 1*).

The results of the chiral GC/MS analysis are given in *Fig. 2*. The configuration and the GC elution order of δ-jasmin lactone were estimated after catalytic hydrogenation of the compound to decano-5-lactone and comparison with the enantiomerically pure, authentic lactone. Both enantiomers were baseline-separated under the chosen GC conditions. The (*S*)-enantiomer was eluted before the (*R*)-isomer. Only the major part of the (*R*)-enantiomer, which can be biosynthesized from the (*S*)-configurated D_6-**7** directly without inversion of configuration (*CIP* rules!), showed full conservation of the label (D_5-**5**). Of the isotopomer D_4-**5**, 89.1% were (*R*)-configurated, whereas the (*R*)/(*S*) ratio of D_3-**5** was estimated to be 34.6:65.4. Calculations over all D-carrying isomers of δ-jasmin lactone resulted in a total (*R*)/(*S*) enantiomer ratio of 88.3:11.7 (*Table 3*). The unlabeled part originated from the endogenous linolenic acid (**4**) was estimated to be almost racemic, possessing a (*R*)/(*S*) enantiomer ratio of 55.3:44.7.

Discussion. – The successful and sufficient synthesis of labeled linolenic acid D_6-**4** was our starting point to investigate the biosynthesis of aroma compounds in *Sporobolomyces odorus*. Three volatile metabolic products of linolenic acid could be identified after administration of D_6-**4** to growing cells of the yeast.

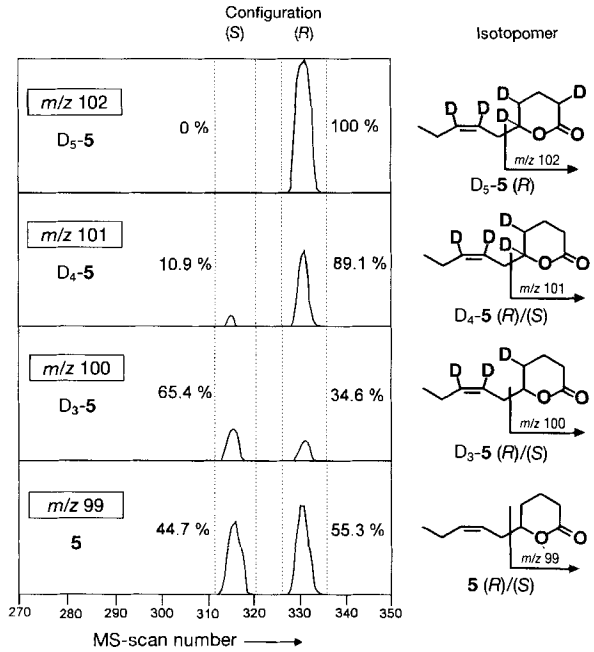


Fig. 2. *Chiral GC/MS analysis of the different isotopomers of the D-labeled δ-jasmin lactone δ-jasmin after administration of (13S,9Z,11E,15Z)-13-hydroxy(²H₆)octadeca-9,11,15-trienoic acid* (D₆-**7**) *to cultures of* S. odorus. Reconstructed ion detection, integration of the signal intensities using the single-ion monitoring mode for the mass fragments *m/z* 99, 100, 101, and 102; for further conditions, see *Exper. Part.*

Table 3. *Relative Distribution of Both Enantiomers of the D-Labeled δ-Jasmin Lactone Isotopomers Formed 102 h after Addition of D₆-7 to Cultures of* S. odorus

	Configuration	
	(*S*)	(*R*)
δ-(²H₅)Jasmin lactone (D₅-**5**)	–	52.5%
δ-(²H₄)Jasmin lactone (D₄-**5**)	3.9%	31.6%
δ-(²H₃)Jasmin lactone (D₃-**5**)	7.8%	4.2%
Total	11.7%	88.3%

One of these compounds was (2*E*,4*Z*)-[²H₃]hepta-2,4-dienoic acid (for the corresponding ester [2,4,5-D]-**8**, see *Fig. 1*), which was detected unlabeled in cultures of *S. odorus* for the first time by *Albrecht* [17]. It cannot be a product of the β-oxidation of linolenic acid, because of its odd-numbered C-chain length. Therefore, a fragmentation of the C_{18}-chain into two odd-numbered fragments could be a suitable explanation for this finding. We do not have further indications of the biochemical pathway to this compound yet.

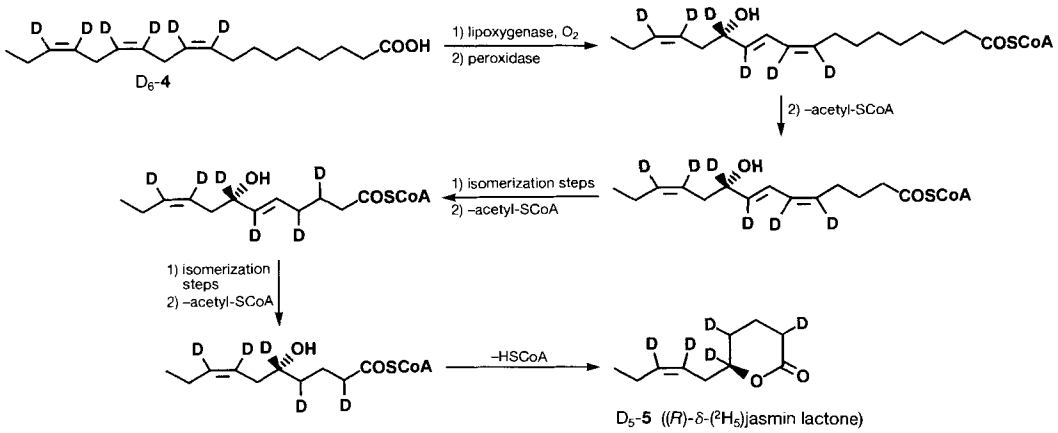
Our results show, that (*Z*,*Z*)-dodeca-6,9-dieno-4-lactone (**6**) is a metabolic product of linolenic acid (**4**). We did not find any metabolic intermediates of the pathway to this

lactone, as we did not for the linoleic acid (**1**) derived analogous compound (*Z*)-dodec-6-eno-4-lactone (**2**), which occurred in the highest concentration of all lactones excreted by *S. odorus* [4]. Therefore, the enzymatic step, which introduces the required OH group at C(10) of linolenic and linoleic acid, remains unclear. Three different enzymatic activities which could catalyze this step can be discussed on the basis of known enzymatic activities so far. These are: *1*) a lipoxygenase/peroxydase system, *2*) an epoxygenase/epoxyhydrolase system, or *3*) a hydratase activity. The main part (84.2%) of D_6-**4**, which was metabolized to dodeca-6,9-dieno-4-lactone, was transformed without loss of D-atoms ($\rightarrow D_6$-**6**). The remaining part had lost one D-atom in the ring moiety of the lactone during its biochemical conversion ($\rightarrow D_5$-**6**). These results correspond to the findings in the biosynthetical study of the analogous (*Z*)-dodec-6-eno-4-lactone, where 92% showed preservation of the complete set of D-atoms [4]. Despite the fact, that we could not determine the enantiomer ratio of the doubly unsaturated lactone **6**, the above presented results reveal limited information about the stereochemistry. In a minimum of 84.2% of this lactone, the unknown initial configuration of the chirality center had been maintained, because subsequent inversion at the stereogenic center would cause an elimination of one D-atom at C(4) of the lactone. Until now, a total inversion of the original configuration was found exclusively for the biosynthesis of decano-5-lactone in *S. odorus*. All other lactones produced by this yeast mainly showed retention of the initial configuration [5] [6] [17] [20]. Previous results demonstrated that the enantiomer composition of the analogous (*Z*)-dodec-6-eno-4-lactone ((*R*)/(*S*) 92:8) and the corresponding labeling patterns fit very well in this line of argumentation [4] [17]. Metabolic degradation of linoleic and linolenic acid to unsaturated-C_{12}-γ-lactones in *S. odorus* appears to follow the same stereochemical course.

The third component which was synthesized from D_6-**4** by *S. odorus* was the important flavor compound δ-jasmin lactone. D_6-**4** as well as D_6-**7** served as precursors in the formation of this compound, yielding almost identical labeling patterns of the produced jasmin lactone (*Table 1*). This supports the assumption that the first step of the biosynthesis of δ-jasmin lactone (**5**) is the (*S*)-stereoselective hydroperoxydation at C(13) of linolenic acid (**4**). Moreover, 88.3% of D_6-**7** were metabolized under retention of the configuration to (*R*)-δ-jasmin lactone (CIP rules!) (*Table 3*). The proposed biochemical pathway to the 5-fold labeled (*R*)-enantiomer D_5-**5**, based on the established β-oxidation pathway of oleic acid, is presented in *Scheme 3* [21–23]. In contrast to these results, the linoleic acid derivative (*S*)-13-hydroxyoctadeca-9,11-dienoic acid was transformed under total inversion of the configuration into enantiomerically pure (*R*)-decano-5-lactone ($> 99\%$; **3**) as former experiments with *S. odorus* showed [5] (see *Scheme 1*). This means, that the stereochemical metabolic course of the (13*S*)-13-hydroxy-fatty-acid degradation is effectively controlled by the presence or absence of the C=C bond at position $\omega - 3$ of the fatty acid.

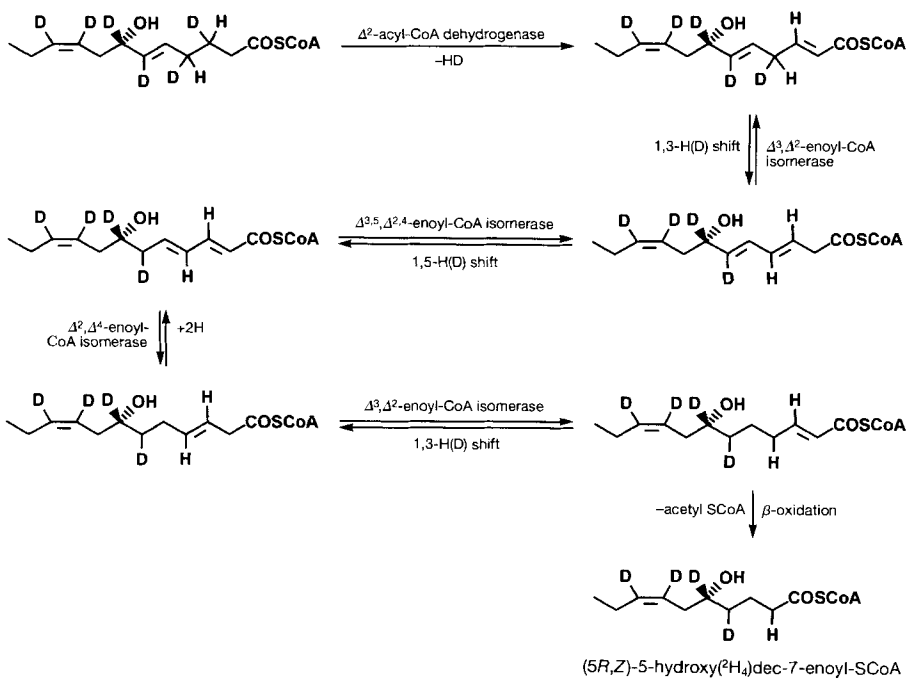
The labeled (*R*)-configurated δ-jasmin lactone contained 31.6% of ($^2H_4$)-labeled D_4-**5**. This loss of one D-atom cannot be explained by inversion at the stereogenic C(5) center of the lactone, caused by an oxidation/reduction step at the OH group bearing C-atom. Therefore, the C=C bond isomerization steps (1,3-H(D) and 1,5-H(D) shifts) during isomerase catalysis of the β-oxidation cycle may be discussed, according to the recently described pathway of fatty acids containing C=C bonds at odd-numbered positions of the C-chain [21–23] (*Scheme 4*). As a limit of our GC/MS method, the

Scheme 3. *Proposed Biosynthetic Pathway of (R)-δ-($^2H_5$)Jasmin Lactone* (D_5-**5**) *from ($^2H_6$)Linolenic Acid* (D_6-**4**)



positions of the label in the lactone cannot be identified. Nevertheless, mechanistic considerations of the pathway into (*R*)-*δ*-jasmin lactone (**5**) lead us to the assumption, that the D-atoms at C(5), C(7), and C(8) of *δ*-jasmin lactone should be conserved, whereas the loss of the D-atom at C(4) may be explained by a single 1,5-H(D) shift and for C(2) by one 1,5-H(D) shift and additional two possible 1,3-H(D) shift isomerization steps.

Scheme 4. *Proposed Isomerization and Chain-Shortening Steps Accompanied by D-Loss in the Formation of (5R,Z)-5-Hydroxydec-7-enoyl-SCoA from (7S,5E,9Z)-7-hydroxydeca-5,9-dienoyl-SCoA*



The formation of the (*S*)-δ-jasmin lactone isotopomers cannot exclusively be explained by the pathway presented in *Schemes 3* or *4*. As previously shown for the conversion of (13*S*,9*Z*,11*E*)-13-hydroxyoctadeca-9,11-dienoic acid, the inversion of the configuration involves an oxidation/reduction step *via* (9*Z*,11*E*,15*Z*)-13-oxooctadeca-9,11,15-trienoic acid. Therefore, a total loss of the D-atom at C(5) of the resulting lactone occurred. This inversion, in which a Δ^{11}-reductase of the C_{18}-precursor may be involved, is comparable to the metabolism of the arachidonic-acid (= (all-*Z*)-eicosa-5,8,11,14-tetraenoic acid) derivatives 12-hydroxyeicosatetraenoic acid in bovine corneal epithelial microsomes and of leukotriene B4 in cultured human hepatoma cells and leukocytes [24] [25]. After the assumed reduction of the C(11)=C(12) bond on the stage of the keto acid, only one further loss of a D-atom is explainable by mechanistic considerations. A H(D)-shift isomerization step can cause the loss of label at C(2) of the lactone. During further metabolic degradation, this pathway leads to (*R*)-3-hydroxyacyl-SCoA, which possesses the required configuration for subsequent β-oxidation in yeasts [26] [27]. More than 90% of the total administered hydroxy fatty acids were obviously metabolized following this pathway, without accumulation of (*S*)-jasmin lactone.

Parallel to the formation of the labeled δ-jasmin lactone, 36.3% of the lactone was biosynthesized without label from endogenous linolenic acid. The enantiomer ratio of the unlabeled δ-jasmin lactone (**5**) was nearly that of a racemic mixture ((*R*)/(*S*) 55.3:44.7). This result was rather unexpected, because, in former experiments, the enantiomer ratios (*R*)/(*S*) of this lactone produced by *S. odorus* was estimated, independently from the actual fermentation conditions, to be constant at 73:27 [17]. Therefore, it must be assumed that the addition of (*S*)-configurated D_6-**7** should have influenced the enzymes which were involved in the control of stereochemistry of δ-jasmin lactone.

In conclusion, the comparison of the biochemical degradation of (*S*)-configurated D_6-**7** with the metabolization of (13*S*,9*Z*,11*E*)-13-hydroxy($^2H_4$)octadeca-9,11-dienoic acid in *S. odorus* demonstrated that both fatty acids are suitable precursors for the formation of δ-jasmin lactone and decano-5-lactone, respectively [5]. However, the stereochemical course of their metabolization was totally different. The stereochemical direction of the reaction was strongly influenced by the C=C bond at the ω–3 position of the fatty acid. During the formation of decano-5-lactone, the initial configuration of the (*S*)-13-hydroxylinoleic-acid derivative was completely inversed, whereas more than 88% of (*S*)-configurated D_6-**7** was transformed into δ-jasmin lactone under retention of the initial configuration.

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Experimental Part

General. All chemicals and solvents were purchased from *Fluka* (Neu-Ulm, Germany), *Aldrich* (Steinheim, Germany), or *Merck* (Darmstadt, Germany), they were of anal. grade, HPLC grade, or otherwise destilled before use. The soybean lipoxygenase was from *Aldrich* (Steinheim, Germany). HPLC: To estimate the enantiomeric purity of D_6-**7**, the compound (96% purity) was first esterified with CH_2N_2 and the ester separated from the remaining stereoisomers by HPLC (250 × 4 mm, 5 µm *Si*, hexan/i-PrOH 99:1; UV detection at 234 nm) and analyzed by HPLC using a chiral stationary phase (250 × 3.8, 5 µm, *Chiralcel OD* (*Daicel*, Japan), hexan/i-PrOH 99:1, UV detection at 234 nm). ^{1}H-NMR Spectra: *Bruker-AMX-500* spectrometer; CDCl as solvent; δ in ppm rel.

to $SiMe_4$ (= 0 ppm), *J* in Hz. Anal. GC/MS (*m/z* (%)): achiral *DB-wax-60W* (60 m × 0.32 mm (i.d.), *J&W*, USA) or chiral *Lipodex-E* (50 m × 0.25 mm (i.d.), *Macherey & Nagel*, Germany) capillary column, coupled either to a flame-ionization detector or connected to a *Varian-CH-5-DF* double-focusing mass spectrometer (*Varian*, Germany); electron ionization (EI) MS (70 eV ionization energy), using a data system *DP 10* (*AMD*, Germany); quantification of the D-label in the lactones by analysis of the D-pattern of the base-fragment ions of δ-lactones (*m/z* 99) and γ-lactones (*m/z* 85) according to a method recently described for γ-lactones [4].

Feeding Experiments and Analysis of Volatiles in the Yeast Cultures. The yeast *Sporobolomyces odorus* (ATCC 24259) was cultivated in 1-l shake flasks containing 200 ml of medium (45 g/l sucrose, 5 g/l lactose, 3 g/l $(MgSO_4 \cdot 7\ H_2O$, 2.5 g/l $(NH_4)_2SO_4$, 2.5 g/l KH_2PO_4, 2.5 g/l L-alanin, 0.1 g/l $CaCl_2 \cdot 2\ H_2O$) at 22° and 80 rpm. To 168 h old cultures, 53 mg (176 μmol, 265 ppm) of D_6-**4** or 50 mg (166.3 μmol, 250 ppm) of D-**7** were added to the culture, followed by 200–300 h of further incubation. To monitor the formation of volatiles, 10 ml of the culture broth were taken at certain time intervals and extracted twice with 60 ml of Et_2O in total. The combined and dried extracts were esterified with diazomethane and subsequently analyzed by GC (*Carlo-Erba-4100* gas chromatograph, *DB Wax 60 W* (60 m × 0.32 mm (i.d.), *J&W*) capillary column).

Separation of the Lactone Fraction and Preparative GC. To obtain larger amounts of the labeled lactones, two 200-ml cultures, grown on the deuterated precursor, were continously extracted for 24 h. The Et_2O extract was carefully concentrated on a 30-cm *Vigreux* column to *ca.* 0.2 ml and then submitted to LC (4.5 g of Al_2O_3/silica gel 2:1), where they were separated into three 40-ml fractions (*Fr. I:* pentane/Et_2O 9:1; *Fr. II:* pentane/Et_2O 1:1; *Fr. III:* Et_2O). *Fr. II* contained the lactones. Further separation of δ-jasmin lactone from accompanying lactones was carried out by prep. GC (*Varian Aerograph 2700*, equipped with a split-outlet; 3.5 m × 4 mm (i.d.) column, packed with 5% *Carbowax 20M* on *Chromosorb WAW/DMCS* 60–80 mesh).

(9,10,12,13,15,16-$^2H_6$)Linolenic Acid (D_6-**4**). Synthesis with modifications according to the methods described in [19] (see *Scheme 2*). ^{1}H-NMR ($CDCl_3$): 0.94 (*t*, *J* = 7, Me); 1.33 (*m*, 8 H, CH_2); 1.65 (*quint.*, *J* = 7, CH_2CH_2COOH); 2.06 (*q* + *t*, *J* = 7, $MeCH_2CD{=}CD$, $CH_2CH_2CD{=}CD$); 2.37 (*t*, *J* = 7, CH_2COOH); 2.83 (br. *s*, 4 H, $CD{=}CDCH_2CD{=}CD$); D-content at olef. C by ^{1}H-NMR: C(9) (97.2%); C(10) (96.3%); C(12) (95.8%); C(13) (97.3%); C(15) (96.2%); C(16) (96.8%). GC/MS (methyl ester): 298 (1, M^+), 297 (< 1, $[M-1]^+$), 267 (2, $[M - MeO]^+$), 240 (3), 227 (1), 195 (2), 153 (4), 125 (12), 112 (33), 99 (48), 83 (100), 74 (13).

(13S,9Z,11E,15Z)-13-Hydroxy(9,10,12,13,15,16-$^2H_6$)octadeca-9,11,15-trienoic Acid (D_6-**7**). To a suspension of 281 mg (1 mmol) of D_6-**4** in 230 ml of 0.1M borate buffer (pH 9.0) under N_2, at 4°, soybean lipoxygenase (20 mg, 160 units) in 5 ml of the buffer was added. Every 2 min, O_2 was bubbled for 15–25 s through the soln. After 10 min, further enzyme (20 mg) was added. The mixture was stirred and gassed with O_2 for another 20 min. The suspension was acidified to pH 4 using 2M H_3PO_4 and extracted with Et_2O (3 × 150 ml), the combined org. phases dried (Na_2SO_4) and evaporated, and the residue containing the hydroperoxy acid directly reduced with $NaBH_4$ (50 mg) in Et_2O (30 ml) at 4° for 5 h. Prep. TLC (200 × 200 × 1 mm, petroleum ether/AcOEt/AcOH 49.5:49.5:1 (*v/v/v*), UV detection at 254 nm) gave 175 mg (60%) of D_6-**7** as a colorless oil, which contained less than 5% of stereoisomers (HPLC). The enantiomeric purity of D_6-**7** was estimated as > 96% by chiral HPLC after esterification with CH_2N_2 and purification by HPLC. D_6-**7**: ^{1}H-NMR ($CDCl_3$): 0.97 (*t*, *J* = 7, Me); 1.28–1.41 (*m*, 8 H, CH_2); 1.63 (*quint.*, *J* = 7, CH_2CH_2COOH); 2.07 (*q*, *J* = 7, $MeCH_2$); 2.18 (*t*, *J* = 7, $CH_2CH_2CD{=}CD$); 2.35 (*m*, $CD{=}CDCH_2CH(OH)$, CH_2COOH); 6.51 (br. *s*, $CD{=}CHCD{=}CD$); D-content at olef. C by ^{1}H-NMR: C(9) (97.9%); C(10) (97.3%); C(12) (96.4%); C(13) (98.0%); C(15) (96.4%); C(16) (96.2%). GC/MS (methyl ester, hydrogenated, and silylated): 377 (< 1, $[M - Me]^+$), 361 (1, $[M - MeO]^+$), 345 (6), 319 (32, $[M - Me_3Si]^+$), 318 (8, $[M - 74]^+$), 215 (6), 176 (100), 73 (98).

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