Metabolism of Deuterated *erythro*-Dihydroxy Fatty Acids in *Saccharomyces cerevisiae*: Enantioselective Formation and Characterization of Hydroxylactones

by **Leif-A. Garbe***, **Katja Morgenthal**[1]), **Katrin Kuscher**[2]), and **Roland Tressl**

Technische Universität Berlin, Institut für Biotechnologie, Molekularanalytik, Seestrasse 13, D-13353 Berlin

(phone: +49-30-45080-231; fax: +49-30-314-27544; e-mail: Leif-A.Garbe@TU-Berlin.de)

Epoxides of fatty acids are hydrolyzed by epoxide hydrolases (EHs) into dihydroxy fatty acids which are of particular interest in the mammalian leukotriene pathway. In the present report, the analysis of the configuration of dihydroxy fatty acids *via* their respective hydroxylactones is described. In addition, the biotransformation of (±)-*erythro*-7,8- and -3,4-dihydroxy fatty acids in the yeast *Saccharomyces cerevisiae* was characterized by GC/EI-MS analysis. Biotransformation of chemically synthesized (±)-*erythro*-7,8-dihydroxy(7,8-$^2H_2$)tetradecanoic acid ((±)-*erythro*-**1**) in the yeast *S. cerevisiae* resulted in the formation of 5,6-dihydroxy(5,6-$^2H_2$)dodecanoic acid (**6**), which was lactonized into (5*S*,6*R*)-6-hydroxy(5,6-$^2H_2$)dodecano-5-lactone ((5*S*,6*R*)-**4**) with 86% ee and into *erythro*-5-hydroxy(5,6-$^2H_2$)dodecano-6-lactone (*erythro*-**8**). Additionally, the α-ketols 7-hydroxy-8-oxo(7-$^2H_1$)tetradecanoic acid (**9a**) and 8-hydroxy-7-oxo(8-$^2H_1$)tetradecanoic acid (**9b**) were detected as intermediates. Further metabolism of **6** led to 3,4-dihydroxy(3,4-$^2H_2$)decanoic acid (**2**) which was lactonized into 3-hydroxy(3,4-$^2H_2$)decano-4-lactone (**5**) with (3*R*,4*S*)-**5** = 88% ee. Chemical synthesis and incubation of (±)-*erythro*-3,4-dihydroxy(3,4-$^2H_2$)decanoic acid ((±)-*erythro*-**2**) in yeast led to (3*S*,4*R*)-**5** with 10% ee. No decano-4-lactone was formed from the precursors **1** or **2** by yeast. The enantiomers (3*S*,4*R*)- and (3*R*,4*S*)-3,4-dihydroxy(3-$^2H_1$)nonanoic acid ((3*S*,4*R*)- and (3*R*,4*S*)-**3**) were chemically synthesized and comparably degraded by yeast without formation of nonano-4-lactone. The major products of the transformation of (3*S*,4*R*)- and (3*R*,4*S*)-**3** were (3*S*,4*R*)- and (3*R*,4*S*)-3-hydroxy(3-$^2H_1$)nonano-4-lactones ((3*S*,4*R*)- and (3*R*,4*S*)-**7**), respectively. The enantiomers of the hydroxylactones **4**, **5**, and **7** were chemically synthesized and their GC-elution sequence on *Lipodex® E* chiral phase was determined.

Introduction. – Epoxides are ubiquitous in the environment, and epoxide hydrolases (EHs) are vital to many organisms through their roles in detoxification, metabolism of epoxides, and processing of signaling molecules. It has been suggested that inhibitors of soluble epoxide hydrolases (sEHs) have therapeutic efficacy in the treatment and management of acute inflammatory diseases [1]. In mammals, sEH metabolizes vasodilatory epoxyeicosatrienoic acids derived from arachidonic acid into their dihydroxy derivatives. Therefore, sEH is hypothesized as a main effector of angiotensin II-induced hypertension [2]. The transformation of oleic acid and linoleic acid epoxides into fruit and dairy product flavors, especially chiral γ-lactones (5-alkyl-

[1]) Current Address: Max-Planck-Institut für Molekulare Pflanzenphysiologie, D-14476 Golm.

[2]) Current Address: Istituto di Ricerca in Biomedicina, Institute for Research in Biomedicine, CH-6500 Bellinzona.

dihydro-2*H*-furanones) which also show bioactivity with pheromone character has been documented in plants and microorganisms [3–6].

Generally, the hydrolysis of epoxides proceeds under inversion of one epoxide C-atom, whereby *cis*- or *erythro*-epoxides yield vicinal *threo*-diols and *trans*- or *threo*-epoxides yield vicinal *erythro*-diols [7]. In contrast, a yeast EH has been characterized converting leukotriene A4 ((5*S*,6*S*)-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid) into (5*S*,6*S*)-dihydroxy-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid, showing retention of the initial epoxide configuration. In the literature, chiral gas chromatographic (GC) separations of *threo*-hydroxylactones have been described [4][5]. Therefore, the absolute configuration of *threo*-3,4-, 4,5- or 5,6-dihydroxy fatty acids can be analyzed by *Lipodex® E* gas chromatography of the respective 3-hydroxy-4-, 5-hydroxy-4-, or 6-hydroxy-5-lactones. In addition, the hydroxylactones themselves are valuable since they show biological activity *e.g.* as mosquito oviposition pheromone [8], as a sex pheromone in the parasitic wasp *Nasonia vitripennis* [9], or may be used as chiral intermediates in natural product synthesis.

To gain more insight into the metabolism of *erythro*-dihydroxy fatty acids and the formation of *erythro*-hydroxylactones, (±)-*erythro*-7,8-dihydroxy(7,8-$^2H_2$)tetradecanoic acid ((±)-*erythro*-**1**), (±)-*erythro*-3,4-dihydroxy(3,4-$^2H_2$)decanoic acid ((±)-*erythro*-**2**), (3*S*,4*R*)-3,4-dihydroxy(3-$^2H_1$)nonanoic acid ((3*S*,4*R*)-**3**)), and (3*R*,4S)-3,4-dihydroxy(3-$^2H_1$)nonanoic acid ((3*R*,4*S*)-**3**) were chemically synthesized, and their biotransformation by *Saccharomyces cerevisiae* was investigated.

Results. – 1. *Chemical Synthesis:* The (±)-*erythro*-7,8-dihydroxy(7,8-$^2H_2$)tetradecanoic acid ((±)-*erythro*-**1**) and (±)-*erythro*-3,4-dihydroxy(3,4-$^2H_2$)decanoic acid ((±)-*erythro*-**2**) were synthesized *via* catalytic deuteration (Pd/$BaSO_4$, $^2H_2$) [6] of tetradec-7-ynoic acid and dec-3-ynoic acid, respectively, followed by OsO_4 dihydroxylation [10] (*Scheme 1*,*a*).

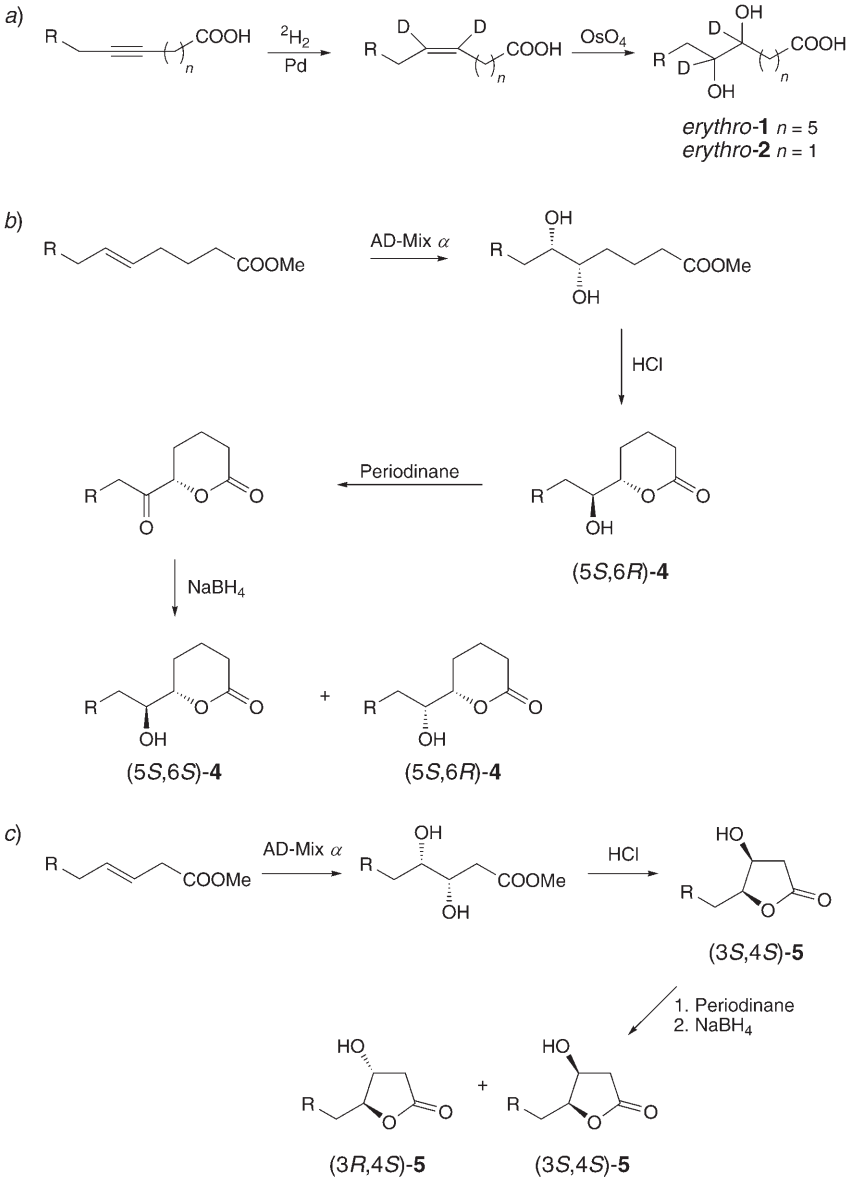
(5*S*,6*S*)-6-Hydroxydodecano-5-lactone ((5*S*,6*S*)-**4**) was synthesized from methyl (5*E*)-dodec-5-enoate by '*Sharpless* asymmetric dihydroxylation' (AD-Mix α) [11] and acid catalyzed lactonization according to [4]. Oxidation (*Dess–Martin* periodinane [12]) of (5*S*,6*S*)-**4** and reduction with $NaBH_4$ yielded an analytical reference containing (5*S*,6*S*)- and (5*S*,6*R*)-**4** (*Scheme 1*,*b*). Analogously, synthesis of (5*R*,6*R*)-**4** using AD-Mix β and subsequent oxidation/reduction yielded a reference mixture of (5*R*,6*R*)- and (5*R*,6*S*)-**4**.

In a similar procedure, the enantiomers (3*R*,4*S*)- and (3*S*,4*R*)-3-hydroxydecano-4-lactone ((3*R*,4*S*)- and (3*S*,4*R*)-**5**) were synthesized from methyl (3*E*)-dec-3-enoate (*Scheme 1*,*c*).

(3*R*,4*S*)- and (3*S*,4*R*)-3,4-dihydroxynonanoic acid ((3*R*,4*S*)- and (3*S*,4*R*)-**3**) were synthesized by '*Sharpless* epoxydation' [13] of (±)-oct-1-en-3-ol. Reaction with L-(+)-tartrate and ^{t}BuOOH yields (2*R*,3*S*)-1,2-epoxyoctan-3-ol (=(1*S*)-1-[(2*R*)-oxiran-2-yl]hexan-1-ol), subsequent treatment with KCN [14] gave (3*R*,4*S*)-3,4-dihydroxynonanenitrile which was subjected to basic saponification resulting in (3*R*,4*S*)-**3** (*Scheme 2*). The enantiomer (3*S*,4*R*)-**3** was synthesized accordingly with D-(−)-tartrate.

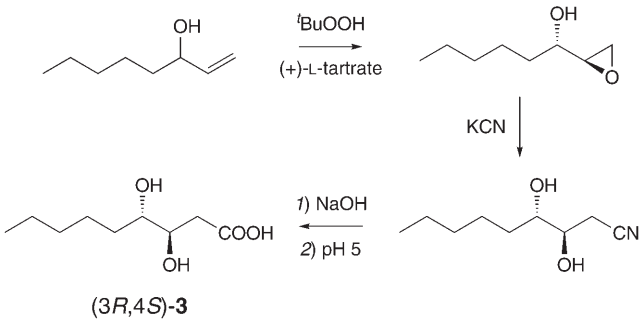
For the synthesis of (3*R*,4*S*)- and (3*S*,4*R*)-3,4-dihydroxy(3-$^2H_1$)nonanoic acid ((3*R*,4*S*)- and (3*S*,4*R*)-**3**), (±)-oct-1-in-3-ol was deuterated with Pd/$BaSO_4$ and $^2H_2$ resulting in (±)-(1,2-$^2H_2$)-oct-1-en-3-ol and further treated as described above.

Scheme 1. *Synthesis of* a) *Deuterated* erythro-*Dihydroxy Fatty Acids*, b) *6-Hydroxydodecano-5-lactone Enantiomers, and* c) *3-Hydroxydecano-4-lactones*



R = $Me(CH_2)_4$–

Scheme 2. *Chemical Synthesis of (3*R,*4*S*)-***3*** via 'Sharpless *Epoxydation'*



However, basic saponification of 3,4-dihydroxy(2,3-$^2H_2$)nonanenitrile caused a complete depletion of the ^2H label at C(2) resulting in the singly deuterated enantiomers of (3-$^2H_1$)-**3**. For the synthesis of (3*R*,4*S*)- and (3*S*,4*R*)-(1-^{13}C)-**3**, the reaction with (2*R*,3*S*)- or (2*S*,3*R*)-1,2-epoxyoctan-3-ol (*Sharpless* epoxidation) was carried out with K^{13}CN (99% ^{13}C). Subsequent saponification gave the enantiomers of *erythro*-(1-^{13}C)-**3**.

The 3,4- and 5,6-dihydroxy acids **3**, **2**, and **6** were lactonized in CH_2Cl_2/0.5M HCl (4:1) into 3-hydroxynonano-4-lactone (**7**, see Formula in *Fig. 1*), 3-hydroxydecano-4-lactone (**5**), and 6-hydroxydodecano-5-lactone (**4**), respectively [15].

2. *Analysis.* 2.1. *GC Separations.* The *erythro*-enantiomers (5*R*,6*S* and 5*S*,6*R*)- of 6-hydroxydodecano-5-lactone (**4**), 3*R*,4*S* and 3*S*,4*R*)- of 3-hydroxydecano-4-lactone (**5**) and of 3-hydroxynonano-4-lactone (**7**), respectively, separated by gas chromatography with a chiral phase (*Lipodex® E*) and the elution sequence was determined (*Table 1*). Enantiomers of 5-hydroxydodecano-6-lactones (**8**) were also separated on *Lipodex® E*, but the absolute configuration could not be assigned. On an achiral phase (polymethylsiloxane phase, *DB-1*), the diastereoisomers of 6-*O*-(trimethylsilyl)-**4** and of 5-*O*-(trimethylsilyl)-**8**, respectively, co-eluted.

Table 1. *Gas Chromatographic* Lipodex® E *Elution Order of Hydroxylactones.* TFA: 6-*O*-trifluoroacetic acid ester, w.d.: without derivatization.

Hydroxylactone	Elution sequence		Derivative
erythro-6-hydroxydodecano-5-lactone (**4**)	1. (5*R*,6*S*)-	2. (5*S*,6*R*)-	TFA
erythro-3-hydroxydecano-4-lactone (**5**)	1. (3*S*,4*R*)-	2. (3*R*,4*S*)-	w.d.
erythro-3-hydroxynonano-4-lactone (**7**)	1. (3*S*,4*R*)-	2. (3*R*,4*S*)-	w.d.

2.2. *EI-MS of* **4**. The *erythro*-6-hydroxydodecano-5-lactones (**4**) showed an EI-MS fragmentation pattern comparable to the previously characterized *threo*-6-hydroxydodecano-5-lactones (**4**) [4]. The ^2H-content at C(6) (*m*/*z* 188/187) and of the lactone ring C(5) (*m*/*z* 173/172) was analyzed by EI-MS of the C(6)-OH trimethylsilyl ethers of **4**: no ^2H depletion could be observed during fermentation of [7,8-$^2H_2$]-**1**.

2.3. *EI-MS of* **7** *and* **5**. The EI mass spectra of the 3-hydroxy-4-lactones unlabeled **7** and [1-^{13}C]-**7**, unlabeled **5** and [3,4-$^2H_2$]-**5**, as well as of the 3-*O*-(trimethylsilyl) ethers of [3-$^2H_1$]-**7** and [1-^{13}C]-**7** are shown in *Figs. 1–3*. The base peak fragment of **7** with *m*/*z* 83

and the abundant peak at *m/z* 101 (*Fig. 1,a*) is in accordance with a rearrangement of the lactone ring under EI conditions, whereby the furan O-atom at the alkyl chain is recorded as $[Me(CH_2)_4CHO]^+$ (*m/z* 101) which eliminated H_2O (neutral loss, *m/z* 83). These fragment ions of **7** do not carry the isotope labels at $^{13}C(1)$ or $^2H-C(3)$, and the label content only exhibits with the molecular ion signals *m/z* 173 and 155 (*Fig. 1,b*). The rearrangement of **7** is confirmed by the EI fragmentation pattern of unlabelled and (3,4-$^2H_2$)-**5**. The abundant fragment ions *m/z* 115 and 97 of unlabelled **5** (*Fig. 2,a*) and the fragmentation pattern of (3,4-$^2H_2$)-**5** with *m/z* 116 and 98 (*Fig. 2,b*) is in accord with the ions $[Me(CH_2)_5C^{(2)}HO]^+$ and subsequent neutral loss of H_2O. The EI mass spectra of *erythro*-**5** and *threo*-**5** [4] are identical.

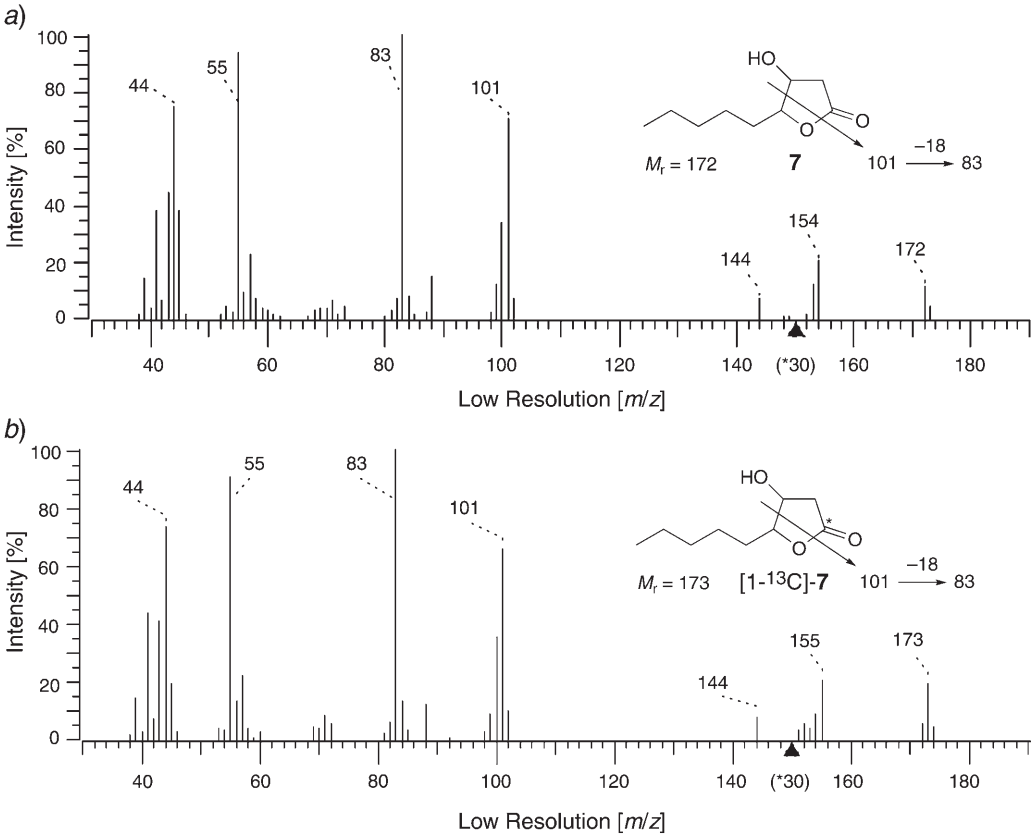


Fig. 1. *EI-MS Spectra of* a) *unlabeled 3-hydroxynonano-4-lactone* (**7**) *and* b) *[1-^{13}C]-***7**

The EI mass spectra of the 3-*O*-(trimethylsilyl) ethers of 3-hydroxy(3-$^2H_1$)nonano-4-lactone ((3-$^2H_1$)-**7**) (*Fig. 3,a*) and ^{13}C-labeled **7** (*Fig. 3,b*) exhibit major MS fragment peaks at *m/z* 117/116 and 102/101 which agree with the increments $[-C(2)H-C(3)^{(2)}HOSiMe_3]^+$ and $[-C(2)H_2C(3)^{(2)}HOSiMe_2]^+$. All 3-*O*-(trimethylsilyl) ethers of 3-hydroxy-4-lactones with different alkyl-chain length exhibit this EI-MS fragmentation pattern. Another rearrangement of 3-*O*-(trimethylsilyl)-(3-$^2H_1$)-**7**

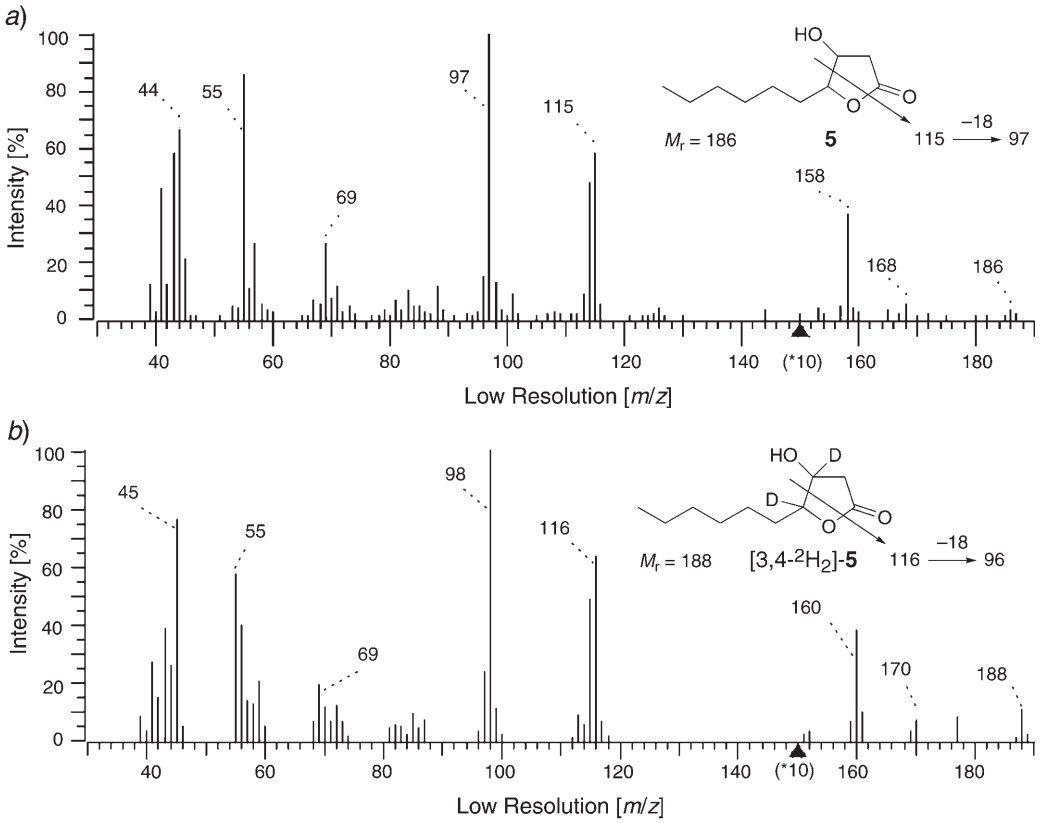


Fig. 2. *EI-MS Spectra of* a) *unlabeled 3-hydroxydecano-4-lactone* (**5**) *and* b) *[3,4-$^2H_2$]-***5**

leads to the fragment ion *m*/*z* 188. Here, the radical cation $M^{+\cdot}$ of 3-*O*-(trimethylsilyl)-(3-$^2H_1$)-**7** eliminates a Me radical from the TMS group, and keten ($H_2C{=}C{=}O$) from the CO group, resulting in the fragment ion *m*/*z* 188 ($M^{+\cdot} - 15 - 42$) which is bearing the O-atoms at C(3) and C(4). The signal *m*/*z* 173 is in accord with the fragment ion $[Me(CH_2)_5CHO{-}SiMe_3]^+$ (*cf. Fig. 3*, *a*). These fragmentations were elucidated also by ^{18}O-labeled isotopomers of **5** (data not shown).

2.4. *EI-MS of* **8**. As stated in *Section 2.1*, *erythro*- and *threo*-5-*O*-(trimethylsilyl) 5-hydroxydodecano-6-lactones (**8**) co-eluted on *DB1* (polymethylsiloxane) GC capillary columns. In contrast to the diastereoisomers of **4**, **5** and **7**, the EI-MS fragment ion intensities of the 5-*O*-(trimethylsilyl) ethers of *erythro*- and *threo*-**8** were not identical (data not shown). Unlabeled 5-*O*-(trimethylsilyl) *threo*-**8** showed fragment ions with *m*/*z* 130 and 201 [4] whereas 5-*O*-(trimethylsilyl) *erythro*-**8** exhibited an EI-MS spectrum similar to the EI-MS of 6-*O*-(trimethylsilyl)-**4** with fragment signals at *m*/*z* 172 and 157.

3. *Incubation Experiments.* All substrates were administered to liquid cultures of the yeast *Saccharomyces cerevisiae* as free acids. Aliquots were taken from the culture broth at indicated times, worked up, derivatized, and analyzed by GC/EI-MS.

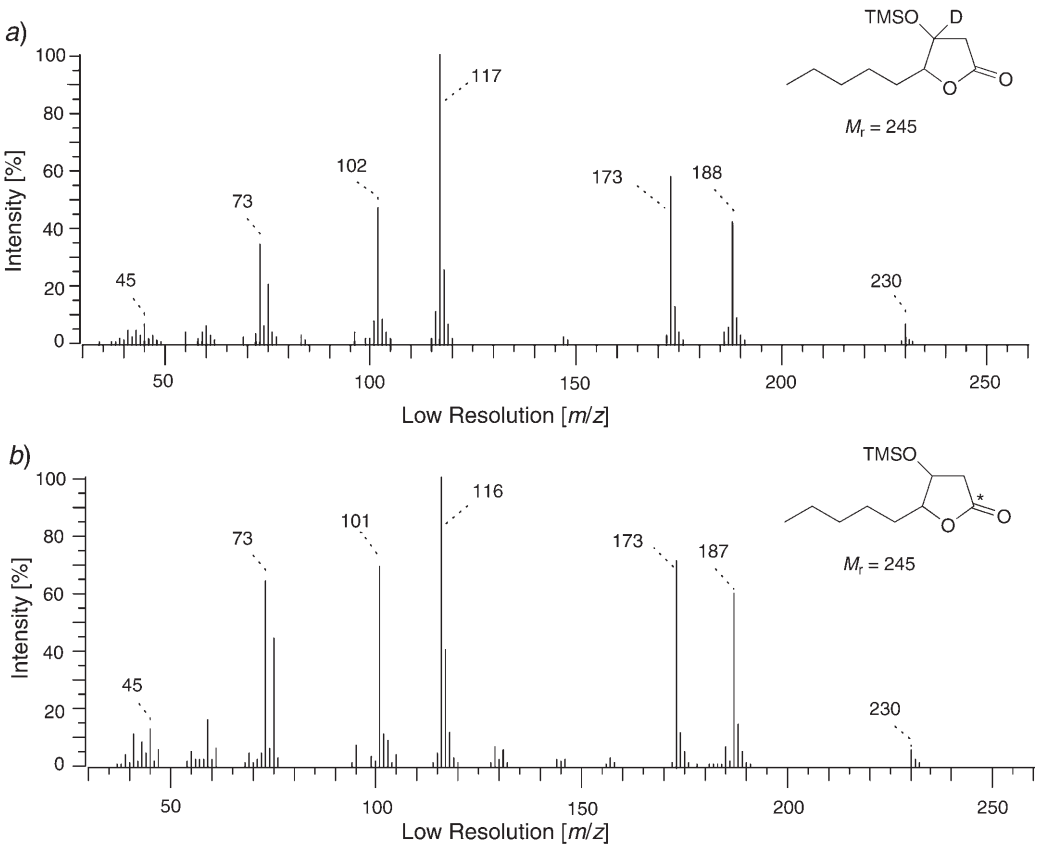


Fig. 3. *EI-MS Spectra of* a) *3*-O-*(trimethylsilyl)-3-hydroxy(3-*$^{2}H_1$*)nonano-4-lactone and* b) *3*-O-*(trimethylsilyl)-3-hydroxy(1-*^{13}C*)nonano-4-lactone*

3.1. *Metabolism of* **1**. The results of the biotransformation of (±)-*erythro*-7,8-dihydroxy(7,8-$^{2}H_2$)tetradecanoic acids (**1**) are summarized in *Table 2* and illustrated in *Fig. 4*. A possible reaction pathway is shown in *Scheme 3*. The substrate was nearly consumed at the end of the fermentation time. During fermentation, two isomeric α-ketols 7-hydroxy-8-oxo(7-$^{2}H_1$)tetradecanoic acid (**9a**) and 8-hydroxy-7-oxo(8-$^{2}H_1$)tetradecanoic acid (**9b**) (EI mass spectra are shown in *Fig. 5*) and hydroxylactones were identified as major products. In contrast to the biotransformation of (±)-*threo*-7,8-dihydroxy(7,8-$^{2}H_2$)tetradecanoic acids by *S. cerevisiae* [4], no decano-4-lactone could be detected as metabolite. The concentration of the α-ketols **9a**/**b** changed during fermentation but neither conversion into *threo*-dihydroxy fatty acids nor depletion of the ^{2}H-content of **1** was observed. The configuration of 6-hydroxydodecano-5-lactones (**4**) was assigned by GC on a chiral phase (*Lipodex® E*) as the 6-acetoxy-**4** derivative. During fermentation, (5*S*,6*R*)-**4** was determined with 86% ee. The *erythro*-5-hydroxydodecano-6-lactones (**8**) separated on *Lipodex® E* and exhibited a similar enantiomeric distribution, but the absolute configuration of enantiomeric **8** could not

Table 2. *Biotransformation of 150 ppm (±)*-erythro-*7,8-Dihydroxy(7,8-$^2H_2$)tetradecanoic Acid* (**1**). Formation of α-ketols **9a/b** and hydroxylactones **4**, **8**, and **5** during fermentation of *S. cerevisiae*[a]) .

Analyte (^{2}H-labeled)	Time [h]			
	72	96	144	264
Substrate				
(±)-*erythro*-**1**	80.6	42.1	43.2	4.0
Metabolites				
Isomeric methyl C_{14}-α-ketols (**9a/b**)	28.5	0.2	35.9	0.1
Methyl 5,6-dihydroxydodecanoate (**6**)	11.8	6.4	10.1	6.3
6-Hydroxydodecano-5-lactone (**4**)	26.2	15.6	30.3	19.4
5-Hydroxydodecano-6-lactone (**8**)	23.3	19.1	21.7	8.0
Methyl 3,4-dihydroxydecanoate (**2**)	0	0	0	2.2
3-Hydroxydecano-4-lactone (**5**)	1.4	0.4	5.6	11.4
Decano-4-lactone	0	0	0	0
Methyl 2-hydroxyoctanoate	0	0	0.2	1.2

[a]) Concentrations in ppm.

be determined. The configuration of *erythro*-3-hydroxydecano-4-lactones (**5**) was assigned by reference enantiomers. The concentration of **5** increased until the end of the fermentation and (3*R*,4*S*)-**5** was formed with 88% ee (*Table 3*).

3.2. *Metabolism of* **2**. Incubation of (±)-*erythro*-3,4-dihydroxy(3,4-$^2H_2$)decanoic acid (**2**) yielded 3-hydroxydecano-4-lactone as major product with low optical purity ((3*S*,4*R*)-**5** = 10% ee). According to the metabolism of *erythro*-**1**, no decano-4-lactone was formed from precursor *erythro*-**2**, and no transformation into *threo*-isomers was observed (*Table 4*).

3.3. *Metabolism of* **3**. Incubation of (3*R*,4*S*)- and (3*S*,4*R*)-3,4-dihydroxy(3-$^2H_1$)nonanoic acids ((3*R*,4*S*)- and (3*S*,4*R*)-**3**), respectively, was performed to investigate differences in the metabolism of the dihydroxy fatty acid enantiomers. Both enantiomers of **3** were comparably degraded by *S. cerevisiae*, and 3-hydroxy(3-$^2H_1$)nonano-4-lactones (**7**) were formed as major products in similar concentrations (*Table 5*). As observed before, no nonano-4-lactone was formed from the precursors (3*R*,4*S*)- and (3*S*,4*R*)-**7** by yeast, respectively.

Discussion. – Naturally occurring unsaturated fatty acids usually exhibit the C=C bond with *cis*-geometry. Oxidations, *e.g.* by epoxygenases [16] or chemically by peracids, lead to *erythro*-epoxides. Generally, the hydrolysis of *erythro*-epoxides proceeds under inversion of the configuration resulting in vicinal *threo*-diols which has been studied, *e.g.*, from soy bean epoxide hydrolases [17]. Therefore, (±)-*erythro*-9,10-epoxy stearic acid [3][6] was transformed *via threo*-diols in fruits and *Sporidiobolus salmonicolor* into aroma active enantiomers of dodecano-4-lactone. The biotransformation of (±)-*threo*-9,10-dihydroxyoctadecanoic acids also lead to dodecano-4-lacton in *S. cerevisiae*, however, only (9*S*,10*S*)-9,10-dihydroxyoctadecanoic acid served as (4*R*)-dodecano-4-lactone precursor whereas the (9*R*,10*R*)-enantiomer did not [4]. As intermediates, *threo*-6-hydroxytetradecano-5-lactones and *threo*-5-hydroxy-tetradecano-6-lactones were identified. These results indicate a more complex degradation

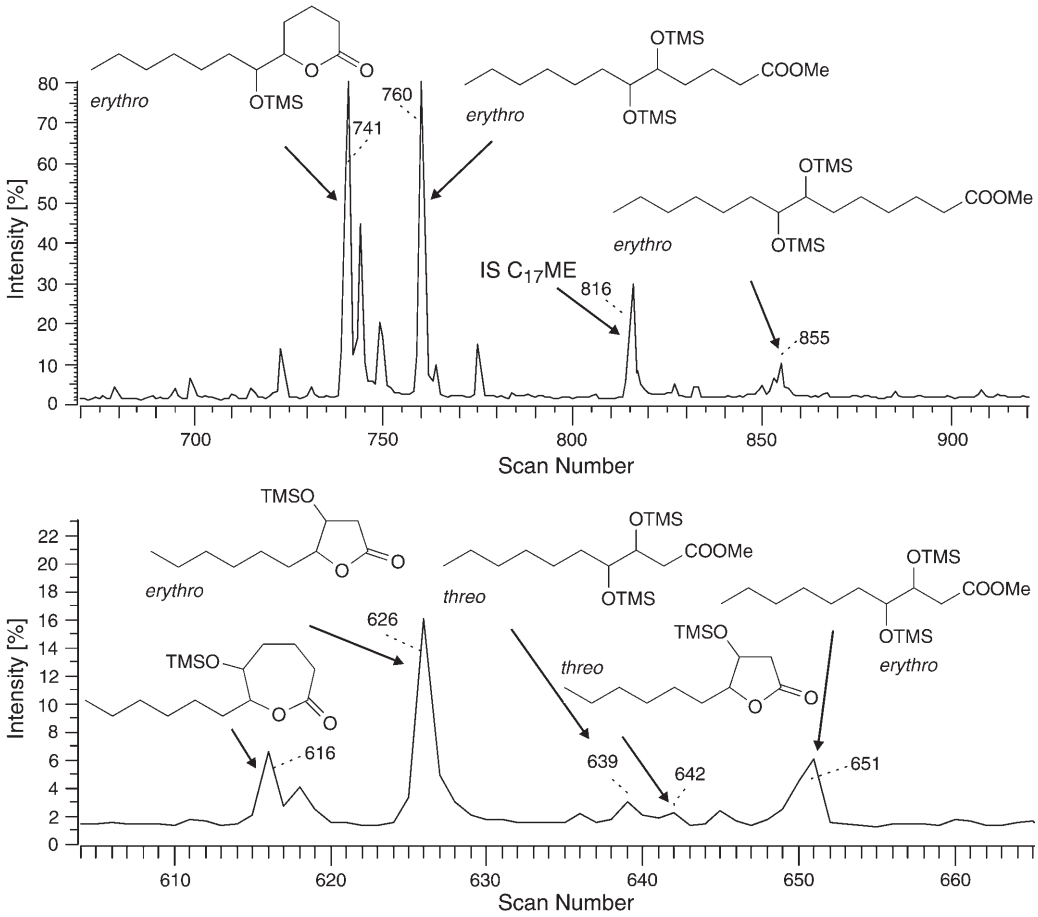
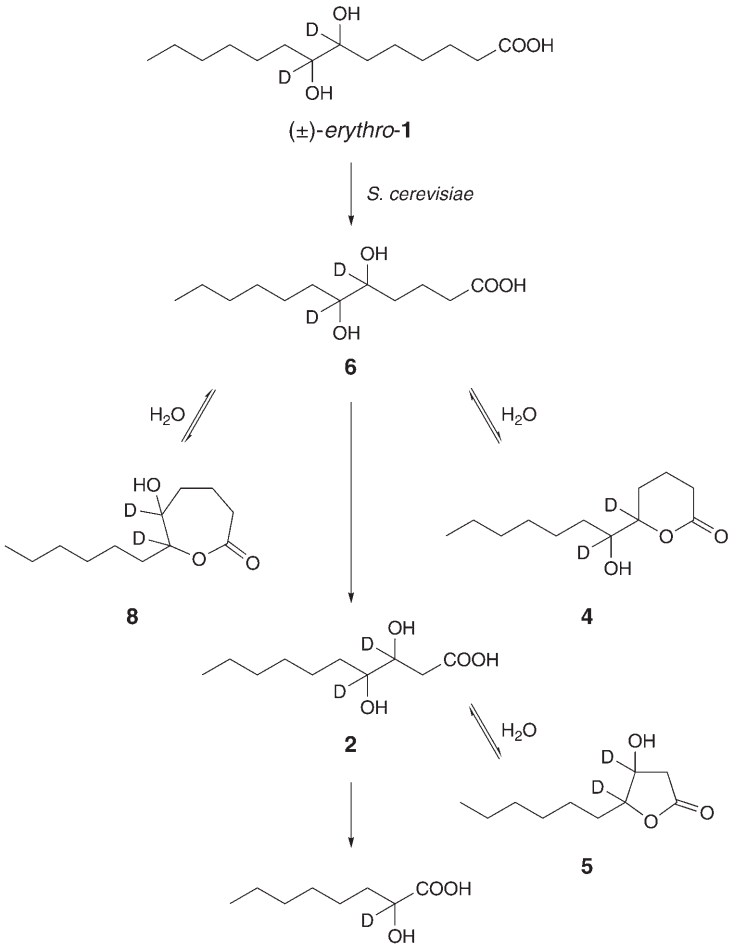


Fig. 4. *GC-Chromatogram* (methylated, silylated, DB-1 stationary GC phase) *of metabolites isolated from the biotransformation of* erythro-*7,8-dihydroxytetradecanoic acid* (**1**) (IS = internal standard: heptadecanoic acid). Traces of (±)-*threo*-3,4-dihydroxydecanoic acid ((±)-*threo*-**2**) and (±)-*threo*-3-hydroxydecano-4-lactone ((±)-*threo*-**5**) were added to emphasize the difference to the *erythro*-diastereoisomers.

pathway in *S. cerevisiae* within γ-lactone synthesis. In addition, *S. cerevisiae* was shown to hydrolyze the *threo*-epoxide leukotriene A4 ((5*S*,6*S*)-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (LTA_4)) into the *threo*-diol (5*S*,6*S*)-dihydroxy-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid, demonstrating retention of the configuration within epoxide hydrolysis [18]. The degradation of *erythro*-6,7-dihydroxydodecanoic acids by *S. cerevisiae* has been reported elucidating differences in their metabolism to *threo*-6,7-dihydroxydodecanoic acids. (±)-*erythro*-6,7-dihydroxydodecanoic acid were transformed into (4*S*,5*R*)- and (4*R*,5*S*)-5-hydroxydecano-4-lactone in a 69 : 31 ratio, whereas (±)-*threo*-6,7-dihydroxydodecanoic acid yielded (4*R*,5*R*)-, (4*S*,5*S*)-, and (4*S*,5*R*)-5-hydroxydecano-4-lactone in a 73 : 19 : 8 ratio. Biotransformation of (6*R*,7*R*)-6,7-

Scheme 3. *Degradation of [7,8-$^2H_2$]-(±)-*erythro-**1** *and Formation of Hydroxylactones* **4**, **8**, *and* **5** *by* S. cerevisiae



dihydroxydodecanoic acid gave (4*R*,5*R*)- and (4*S*,5*R*)-5-hydroxydecano-4-lactone in a 78 : 22 ratio [5]. These results confirm a conversion of the *threo*-isomer into the *erythro*-isomer and the predominant lactonization of the (4*S*,5*R*)-5-hydroxydecano-4-lactone.

Therefore, the degradation of (±)-*erythro*-7,8-dihydroxy(7,8-$^2H_2$)tetradecanoic acid ((±)-*erythro*-**1**) and (±)-*erythro*-3,4-dihydroxy(3,4-$^2H_2$)decanoic acid ((±)-*erythro*-**2**), respectively, was investigated in yeast under biotransformation conditions. The main metabolites were 3-hydroxydecano-4-lactone (**5**), 5-hydroxydodecano-6-lactone (**8**), and 6-hydroxydodecano-5-lactone (**4**) as well as isomeric α-ketols. The hydoxylactone structures were confirmed by chemical synthesis, their EI-MS fragmentations were elucidated by means of isotopically labeling (^2H and ^{13}C), and their elution order by gas chromatography with a chiral phase (*Lipodex® E*) was proven by synthetic enantiomers (except 5-hydroxydodecano-6-lactones (**8**)). These hydroxylactones can

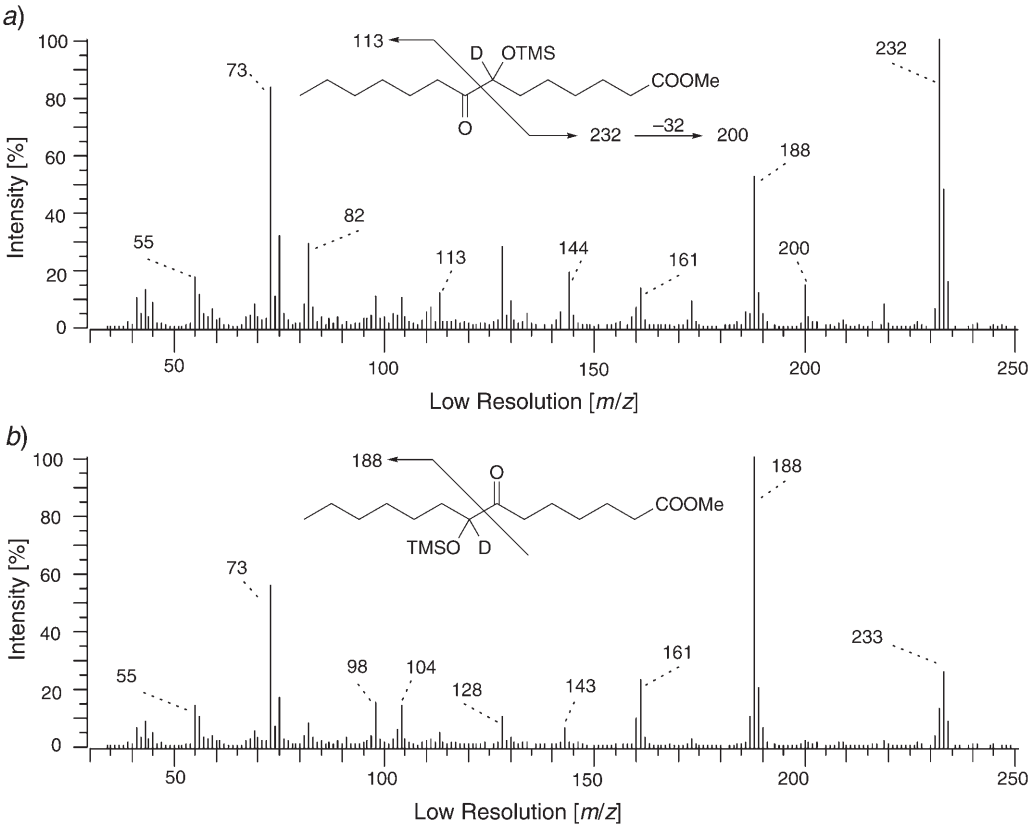


Fig. 5. *Postulated EI-MS spectra of α-ketols a*) *7-hydroxy-8-oxo(7-$^2H_1$)tetradecanoic acid* (**9a**) *and of b*) *8-hydroxy-7-oxo(8-$^2H_1$)tetradecanoic acid* (**9b**) (methylated, silylated) *isolated from cultures of* S. cerevisiae *after incubation of* (±)-erythro-*7,8-dihydroxy(7,8-$^2H_2$)tetradecanoic acid* (**1**)

Table 3. *Isotopomeric and Enantiomeric 6-Hydroxydodecano-5-lactone* (**4**) *and 3-Hydroxydecano-4-lactone* (**5**) *Isolated from Liquid Cultures of* S. cerevisiae *after Incubation of 150 ppm* (±)-erythro-*7,8-Dihydroxy(7,8-$^2H_2$)tetradecanoic Acid* (**1**)

	(5*R*,6*S*)-**4** [%]	(5*S*,6*R*)-**4** [%]	(3*R*,4*S*)-**5** [%]	(3*S*,4*R*)-**5** [%]
[5,6-$^2H_2$]-**4**	14	86		
[3,4-$^2H_2$]-**5**			88	12

serve as chiral building blocks and exhibit bioactivity themselves. The *erythro*-6-hydroxy-5-lactones are of particular interest, (5*R*,6*S*)-6-acetoxyhexadecan-5-olide is the major component of the mosquito oviposition attractant pheromone [8], and its chemical synthesis was performed utilizing the '*Sharpless* asymmetric epoxydation', as described previously [19]. In addition, hydroxylactones with anti-tumor and anti-malarial activities have been published [20]. The lactonization of *erythro*-3-hydroxy-decano-4-lactone proceeded with favor to the (3*S*,4*R*)-enantiomer starting from

Table 4. *Biotransformation of 150 ppm (±)*-erythro-*3,4-Dihydroxy(3,4-$^2H_2$)decanoic Acid* (**2**). Formation of 3-hydroxydecano-4-lactone (**5**)[a])[b]) during fermentation of *S. cerevisiae*.

Analyte (^{2}H-labeled)	Time [h]			
	24	48	144	312
Substrate: (±)-*erythro*-**2**	8.7	3.5	2.7	8.6
Metabolite: 3-Hydroxydecano-4-lactone (**5**)	23.9	16.0	5.4	13.2

[a]) Concentrations in ppm. [b]) (3*R*,4*S*)-**5**:(3*S*,4*R*)-**5** = 45:55.

Table 5. *Biotransformation of 150 ppm (3*R*,4*S*)- and of 150 ppm (3*S*,4*R*)-3,4-Dihydroxy(3-$^2H_1$)nonanoic Acid* (**3**). Formation of 3-hydroxynonano-4-lactone (**7**)[a]) during fermentation of *S. cerevisiae* after 144 h incubation time.

Analyte (^{2}H-labeled)	(3*R*,4*S*)-[3-$^2H_1$]-**3**	(3*S*,4*R*)-[3-$^2H_1$]-**3**
Substrate (3*R*,4*S*)-**3** or (3*S*,4*R*)-**3**	5.2	7.2
Metabolites		
3-Hydroxynonano-4-lactone (**7**)	16.8	26.9
Nonano-4-lactone	0	0

[a]) Concentrations in ppm.

substrates **2** and **3**. Using the '*Fischer* projection', the configuration of (3*S*,4*R*)-**5** and of (4*S*,5*R*)-5-hydroxydecano-4-lactone at C(4) is D. *S. cerevisiae* might prefer lactonization of *erythro*-diols with D-configuration at C(4). However, biotransformation of **1** ended up with (3*R*,4*S*)-3-hydroxydecano-4-lactone (**5**) exhibiting L-configuration at C(4), and therefore the inverse configuration was formed with 88% ee. Within this biotransformation of **1**, (5*S*,6*R*)-6-hydroxydodecano-5-lactone (**4**) was analyzed with 86% ee, thus eliminating higher concentrations of (5*S*,6*R*)-5,6-dihydroxydodecanoic acid (**6**) from the metabolism *via* β-oxidation. The yeast β-oxidation exclusively occurs inside the peroxisomes and the enzyme cascade degrades D-3-hydroxyacylCoA, whereas L-3-hydroxyacylCoA has to be metabolized alternatively [21]. The D-configuration at C(3) of (3*S*,4*R*)-3,4-dihydroxydecanoic acid (**2**) accords with the β-oxidation stereoselectivity. Therefore, we assume the accumulation of (3*R*,4*S*)-3,4-dihydroxydecanoic acid during transformation of **1** finally leading to (3*R*,4*S*)-3-hydroxydecano-4-lactone (**5**). Since the configuration of the dihydroxy acids cannot be determined by GC on chiral phases, their configuration can only be postulated by analyzing the hydroxylactones. The stereoselective synthesis of chiral hydroxylactones from (±)-*erythro*-dihydroxy fatty acids by *S. cerevisiae* is of preparative interest because the precursors are easily available by catalytic OsO_4 dihydroxylation of natural *cis*-configured fatty acids.

Additionally to the characterization of hydroxylactones, a main focus was the disclosure of the metabolic fate of *erythro*-dihydroxy fatty acids. During fermentation, isomeric α-ketols were identified, but, no degradation products were characterized. Generally, ketones can undergo a *Baeyer–Villiger* oxidation with peracids, and fatty acid metabolism in yeasts proceeds inside the peroxisomes where H_2O_2 is present. The genome of the yeast *Saccharomyces cerevisiae* was unsuccessfully screened for a DNA

sequence that is comparable to the bacterial *Baeyer–Villiger* monooxygenase (BVMO) encoding gene. Therefore, a bacterial BVMO gene was overexpressed in *S. cerevisiae* [22]. However, the yeast *Sporobolomyces odorus* endogenously metabolizes the γ- and δ-lactone precursors 4- and 5-hydroxy decanoic acids *via* 4- and 5-oxodecanoic acids, which are further metabolized by a *Baeyer–Villiger* type oxidation. The *Baeyer–Villiger* esters have been characterized [23] and we assume a comparable metabolic fate of the α-ketols **9a/b**.

Experimental Part

1. *General.* LC: Liquid chromatography; GC/EI-MS: Gas chromatography/electron impact mass spectrometry: fused silica *DB-1* cap. column (poly(dimethylsiloxan)), 60 m × 0.32 mm i.d., 0.25 μm film (*J&W Scientific*, Folsome, CA), temp. program 4 min at 80°, then 4°/min to 280°, 100 kPa He (4.0); or *Lipodex® E* (octakis-(3-*O*-butyryl-2,6-di-*O*-pentyl-)-γ-cyclodextrin, 50 m × 0.25 i.d., 0.25 μm film *(Macherey und Nagel*, D-Düren)), temp. program 10 min at 70°, then 4°/min to 220°, 100 kPa He (4.0); *Carlo Erba-Fractovap 4160* coupled by a heated transfer line to a double focusing electron impact (EI) ionization *Varian-Mat 8230* mass spectrometer, ionization energy 70 eV; *m/z* (intensity in %). ^{1}H- and ^{13}C-NMR spectra: *AMX-500* spectrometer (*Bruker*, D-Karlsruhe); chemical shifts δ in ppm rel. to Me_4Si (0 ppm) as external standard, *J* in Hz, signal assignment by H,H-COSY and H,C-HETCOR.

2. *Dihydroxy Fatty Acids.* 2.1. *(±)*-erythro-*7,8-Dihydroxy(7,8-$^2H_2$)tetradecanoic Acid* (*(±)*-erythro-**1**). As described for *erythro*-6,7-dihydroxy(6,7-$^2H_2$)dodecanoic acid in [5]. 1,5-Dibromopentane (46 g, 0.2 mol) was coupled with lithiated oct-1-yne, and the resulting 1-bromotridec-6-yne was substituted with NaCN (19.6 g, 0.4 mol) to give tetradec-7-ynenitrile (9.2 g, 45 mmol) in a 45% overall yield. Saponification of the nitrile with Na_2O_2 afforded tetradec-7-ynoic acid which was hydrogenated (Pd 5% on $BaSO_4$) with $^2H_2$ gas to give (7*Z*)-(7,8-$^2H_2$)tetradec-7-enoic acid. Dihydroxylation using OsO_4 yielded (±)-*erythro*-7,8-dihydroxy(7,8-$^2H_2$)tetradecanoic acid ((±)-erythro-**1**, 5.5 g, 25 mmol, 55% yield based on tetradec-7-ynenitrile). Mass spectra of the intermediates were identical to the data of *threo*-**1** in the literature [4]. ^{1}H-NMR ($CDCl_3$): 3.21–3.38 (*m*, H–C(7), H–C(8), 0.16 H, *i.e.* 92% ^{2}H-labeling); 2.22 (*t*, *J* = 7, C*H*$_2$COOH); 1.05–1.51 (*m*, CH_2, 18 H); 0.81 (*t*, *J* = 7, Me). GC/MS: 405 (3, $[M - Me]^+$), 305 (30, $[232 + TMS]^+$), 232 (100), 188 (90), 147 (20), 128 (20), 73 (80).

2.2. *(±)*-erythro-*3,4-Dihydroxy(3,4-$^2H_2$)decanoic Acid* (*(±)*-erythro-**2**). As described for (±)-*threo*-3,4-dihydroxy(3,4-$^2H_2$)decanoic acids in [4]. In brief, but-3-yn-1-ol and dihydropyran were coupled to 2-(but-3-ynyloxy)tetrahydropyran which was lithiated (BuLi) and coupled with 1-bromohexane in DMPU to 2-(decynyloxy)tetrahydropyran. The THP ether was cleaved with TsOH in MeOH to dec-3-yn-1-ol. *Jones* oxidation (CrO_3/H_2SO_4) of dec-3-yn-1-ol to dec-3-ynoic acid and subsequent hydrogenation (Pd, $BaSO_4$) with $^2H_2$ gas yielded (3*Z*)-(3,4-$^2H_2$)dec-3-enoic acid (1.21 g, 7 mmol, 15% based on 3-butyn-1-ol). According to the synthesis of (±)-*erythro*-**1**, 1.21 g (3*Z*)-(3,4-$^2H_2$)dec-3-enoic was dihydroxylated with stoichiometric amounts of OsO_4 in ^{t}BuOOH. LC of the crude dihydroxy acid gave (±)-*erythro*-3,4-dihydroxy(3,4-$^2H_2$)decanoic acid ((±)-*erythro*-**6**, 0.7 g 3.4 mmol, 49%). ^{1}H-NMR ($CDCl_3$): 3.29–3.38 (*m*, H–C(3), H–C(4), 0.10 H, *i.e.* 95% ^{2}H-labeling); 2.43 (br. *s*, CH_2(2)); 1.15–1.60 (*m*, CH_2(5) to CH_2(9)); 0.83 (*t*, *J* = 7, Me).

2.3. *Asymmetric Dihydroxylation* (AD). Formation of methyl *threo*-3,4-dihydroxydecanoates and methyl *threo*-5,6-dihydroxydodecanoates. AD was performed by the '*Sharpless* AD' method as described essentially in [5]. Methyl (*E*)-alkanoates yielded (*S,S*)-dihydroxy fatty acid methyl esters with 'AD-Mix α' and *vice versa*.

*Methyl (3*E*)-Dec-3-enoate.* Under N_2, malonic acid (propanedioic acid, 10.4 g, 0.1 mol) was dissolved in dry DMSO (50 ml), and octanal (6.4 g, 50 mmol) was added. Subsequently, a freshly prepared soln. of piperidine (85 mg, 1 mmol) and AcOH (60 mg, 1 mmol) in 0.5 ml dry DMSO was added and stirred for 20 min at r.t. The mixture was heated to 85° for 4 h, cooled to r.t., poured on 100 ml ice water and extracted three times with Et_2O (each 50 ml). The org. phase was washed (H_2O), dried (Na_2SO_4), and evaporated. The crude (3*E*)-dec-3-enoic acid was subjected to methylation (HCl/MeOH)

and purification by LC (100 g SiO_2, petroleum ether (PE)/ethyl acetate (AcOEt) 90:10) yielded methyl (3*E*)-dec-3-enoate (7.4 g 40 mmol, 80% based on octanal) similar to products reported in [4].

*Methyl (5*E*)-dodec-5-enoate.* The synthesis has been reported previously [4].

2.4. *(3*R,*4*S*)- and (3*S,*4*R*)-3,4-Dihydroxy(1-^{13}C)nonanoic acid* (*(3*R,*4*S*)*-**3** *and (3*S,*4*R*)*-**3**). *1,2-Epoxyoctan-3-ol* (*= 1-(oxiran-2-yl)hexan-1-ol*). At $-20°$, $Ti(O^iPr)_4$ (14.3 g, 50 mmol), (+)-L- or (−)-D-diethyl tartrate (12.4 g, 60 mmol) and oct-1-en-3-ol (6.3 g, 50 mmol) were stirred in 150 ml abs. CH_2Cl_2, and abs. *tert*-butyl hydroperoxide (2.7 g, 30 mmol) was added. The homogenous soln. was stored at $-21°$ for 21 d. For work up, acetone ($-21°$, 350 ml) and H_2O (15 ml) were added and the mixture was stirred until r.t. was reached. The mixture was centrifuged (5000 × g), the supernatant was chilled to $-20°$, stirred, and a soln. of tartaric acid (10% in H_2O, 125 ml) was added slowly and warmed up to r.t. during 90 min. The phases were separated and the org. phase was washed (H_2O, 100 ml), dried (Na_2SO_4), and evaporated. The resulting oil was dissolved in Et_2O (375 ml), and at 0°, 1M NaOH (150 ml) was added and the mixture was stirred for 30 min. The phases were separated, and the Et_2O phase was washed three times with brine (each 50 ml), evaporated and purified by LC (50 g SiO_2, 500 ml PE/AcOEt (18:2)): 2.8 g (80%) of (1*S*)-1-[(2*R*)-oxiran-2-yl]hexan-1-ol (from (+)-L-diethyl tartrate) and 2.7 g (78%) of (1*R*)-1-[(2*S*)-oxiran-2-yl]hexan-1-ol (from D-(−)-diethyl tartrate) were isolated, resp., as well as the remaining enantiomers (3*R*)- and (3*S*)-oct-1-en-3-ol (data not shown). GC/MS: 144 (1, $M^{+\cdot}$), 101 (15), 83 (80), 73 (35), 57 (30), 55 (100), 45 (30), 43 (40), 41 (45).

erythro-*3,4-Dihydroxy(1-^{13}C)nonanoic Acid:* At 20°, (1*S*)-1-[(2*R*)-oxiran-2-yl]hexan-1-ol or (1*R*)-1-[(2*S*)-oxiran-2-yl]hexan-1-ol (500 mg, 7.5 mmol) were dissolved in 15 ml MeOH, $K^{13}CN$ (0.5 g, 7.5 mmol) were added, and the mixture was stirred overnight. The solvent was evaporated, the residue dissolved in H_2O (10 ml) and acidified at 0° with 1M HCl. The residue was extracted twice with Et_2O (each 50 ml), dried (Na_2SO_4), and evaporated, whereby (2*R*,3*S*)- or (2*S*,3*R*)-3,4-dihydroxy(1-^{13}C)nonanenitrile was isolated: *3,4-dihydroxy-(1-^{13}C)nonanoic acid nitrile* (3-*O*-,4-*O*-bis-trimethylsilylether). GC/MS: 228 (5), 210 (15), 187 (5), 173 (25), 144 (15), 143 (18), 142 (10), 116 (100), 101 (40), 75 (70), 73 (60), 55 (20), 45 (20), 43 (20), 41 (20).

The crude nitrile was dissolved in H_2O (5 ml) and NaOH (0.6 g, 15 mmol) and stirred for 24 h. The mixture was heated to 80° and a moderate N_2 stream was bubbled through the soln. until no NH_3 was detected. The mixture was acidified with 1M H_3PO_4 to pH 5, extracted three times with Et_2O (each 30 ml), and purified by LC (50 g SiO_2, 500 ml PE/AcOEt/AcOH (15:5:0.2)): 200 mg (30% based on the epoxide) (3*R*,4*S*)-3,4-dihydroxy(1-^{13}C)nonanoic acid ((3*R*,4*S*)-**3** and 210 mg (32%) (3*S*,4*R*)-3,4-dihydroxy(1-^{13}C)nonanoic acid (3*S*,4*R*)-**3**).

*(3*R,*4*S*)- and (3*S,*4*R*)-3,4-Dihydroxy(3-$^2H_1$)nonanoic Acid.* The deuterated enantiomers of *erythro*-3,4-dihydroxynonanoic acids were synthesized according to the [1-^{13}C]-labeled compounds. (1,2-$^2H_2$)-oct-1-en-3-ol was synthesized by hydrogenation (Pd ($BaSO_4$), $^2H_2$) of oct-1-yne-3-ol. *(1,2-$^2H_2$)-oct-1-en-3-ol:* GC/MS: 112 (2, [$M - H_2O^+$), 101 (3), 99 (4), 83 (7), 74 (15), 60 (20), 59 (100), 58 (25), 43 (30), 41 (20).

*Methyl (3*R,*4*S*)-3,4-Dihydroxy[3-$^2H_1$]nonanoate* (bis(trimethylsilyl) ether). GC/MS: 332 (1, [$M - 15$]$^+$), 318 (3), 249 (25), 244 (5), 243 (5), 173 (75); 147 (20), 103 (29), 73 (100), 45 (10).

3. *Hydroxylactones.* 3,4- And 5,6-dihydroxy fatty acids or their corresponding methyl esters (1–100 mg) were lactonized in 0.5M HCl/CH_2Cl_2 overnight at 20°. The phases were separated, the CH_2Cl_2 phase was neutralized ($NaHCO_3$), dried (Na_2SO_4), and evaporated.

*(3*S,*4*R*)- and (3*R,*4*S*)-3-Hydroxydecano-4-lactone* (*(3*S,*4*R*)*- and *(3*R,*4*S*)*-**5**). The enantiomers (3*R*,4*R*)- and (3*S*,4*S*)-**5** were oxidized at OH–C(3) with '*Dess–Martin* periodinane' [12] to yield enantiomers (4*R*)- and (4*S*)-3-oxodecano-4-lactone, resp., which were reduced with $NaBH_4$ in Et_2O to give (3*R*,4*R*)/(3*R*,4*S*)-**5** and (3*S*,4*S*)/(3*S*,4*R*)-**5**, resp. Accordingly, (5*R*,6*R*)- and (5*S*,6*S*)-6-hydroxydodecano-5-lactones (**4**) were oxidized into (5*R*)- and (5*S*)-6-oxododecano-5-lactones, resp., and $NaBH_4$ reduced to (5*R*,6*R*)/(5*R*,6*S*)-**4** and (5*S*,6*S*)/(5*S*,6*R*)-**4**, resp. The diastereomeric lactones could be separated by LC (SiO_2, PE/AcOEt 75:25).

(±)-erythro-*6-Hydroxydodecano-5-lactone* (**4**). ^{1}H-NMR ($CDCl_3$): 4.18–4.24 (*m*, H–C(5)); 3.72–3.80 (*m*, H–C(6); 2.52–2.58 (*m*, H_a–C(2)); 2.36–2.44 (*m*, H_b–C(2)); 1.68–1.97 (*m*, CH_2(3), CH_2(4)); 1.40 (*q*, *J* = 7, CH_2(7)); 1.18–1.32 (*m*, CH_2, 8 H); 0.83 (*t*, *J* = 7, Me). ^{13}C-NMR: 171.93 (C(1)); 83.51 (C(5)); 72.19 (C(6)); 31.64 (C(7)); 31.63; 29.70 (C(2)); 29.12; 25.80; 22.50; 21.04 (C(4)); 18.22 (C(3)); 13.99 (C(12)).

(3S,4R)-3-Hydroxy(1-^{13}C)nonano-4-lactone (*(3S,4R)*-**7**): ^{1}H-NMR ($CDCl_3$): 4.27 – 4.35 (*m*, H–C(4)); 4.16 – 4.24 (*m*, H–C(3)); 2.42 – 2.49 (*m*, H_a–C(2)); 2.71 – 2.83 (*m*, H_b–C(2)); 1.47 – 1.62 (*m*, CH_2(5)); 1.30 – 1.46 (*m*, CH_2(6)); 1.20 – 1.30 (*m*, CH_2, 4 H); 0.89 (*t*, *J* = 6.5, Me). ^{13}C-NMR: 176.3 (C(1)); 88.40 (C(4)); 71.18 (C(3)); 37.43 (*d*, *J* = 49 (C(2)); 32.78 (C(5)); 31.26; 24.75; 22.29 (C(6)); 13.82 (C(9)).

4. *Yeast Strain, Culture Conditions, Sampling and Work Up*. Yeast (*Saccharomyces cerevisiae* IfG 06136) was obtained from the culture collection of the 'Institut für Gärungstechnologie', Berlin. The organism was stored (4°) on Wort agar-agar slants. For metabolic experiments cells were cultivated in a medium (200 ml) consisting of 6.0 g/l glucose, 3.0 g/l $MgSO_4$, 2.5 g/l $(NH_4)_2SO_4$, 2.5 g/l KH_2PO_4, 2.5 g/l L-alanine and 3.0 g/l yeast extract (pH 5.5) on a horizontal shaker at 17° and 100 rpm. The pre-culture (10 ml) was inoculated into 200 ml fresh culture medium and 30 mg (±)-*erythro*-7,8-dihydroxy(7,8-$^2H_2$)tetradecanoic acid ((±)-*erythro*-**1**), 30 mg (±)-*erythro*-3,4-dihydroxy(3,4-$^2H_2$)decanoic acid ((±)-*erythro*-**2**), 30 mg (3*R*,4*S*)-3,4-dihydroxy(3-$^2H_1$)nonanoic acid ((3*R*,4*S*)-**3**), or 30 mg (3*S*,4*R*)-3,4-dihydroxy(3-$^2H_1$)nonanoic acid ((3*S*,4*R*)-**3**), each in 250 µl EtOH were immediately administered to different flasks. At certain times (24, 48, 72, 96, 120, 144, 168, 216, 268, 312 h), aliquots (10 ml) of the culture broth were taken, internal standards (5 ppm of 1-decanol, δ-octalactone and heptadecanoic acid) were added, extracted with Et_2O (2 × 20 ml), dried (Na_2SO_4), methylated with diazomethane and converted into derivatives (silyl-ether or trifluoroacetyl-ester) under standard conditions [24] if necessary.

REFERENCES

[1] K. R. Schmelzer, L. Kubala, J. W. Newman, I.-H. Kim, J. P. Eiserich, B. D. Hammock, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 9772.
[2] O. Jung, R. P. Brandes, I.-H. Kim, F. Schweda, R. Schmidt, B. D. Hammock, R. Busse, I. Fleming, *Hypertension* **2005**, *45*, 759.
[3] M. Schöttler, W. Bohland, *Helv. Chim. Acta* **1996**, *79*, 1488.
[4] L.-A. Garbe, R. Tressl, *Helv. Chim. Acta* **2004**, *87*, 180.
[5] L.-A. Garbe, R. Tressl, *Helv. Chim. Acta* **2003**, *86*, 2349.
[6] T. Haffner, R. Tressl, *Lipids* **1998**, *33*, 47.
[7] S. S. Gill, B. D. Hammock, *Biochem. Biophys. Res. Commun.* **1979**, *89*, 965.
[8] B. R. Laurence, J. A. Pickett, *J. Chem. Soc., Chem. Commun.* **1982**, 59.
[9] J. Ruther, L. M. Stahl, S. Steiner, L. A. Garbe, T. Tolasch, *J. Exp. Biol.* **2007**, *210*, 2163.
[10] M. Hamberg, R. P. Herman, U. Jacobsson, *Biochim. Biophys. Acta – Lipids and Lipid Metabolism* **1986**, *879*, 410.
[11] R. A. Johnson, K. B. Sharpless, in 'Catalytic Asymmetric Synthesis', Ed. I. Oijama, Wiley VCH, New York, 1993, p. 227.
[12] D. B. Dess, J. C. Martin, *J. Org. Chem.* **1983**, *48*, 4155.
[13] T. Katsuki, K. B. Sharpless, *J. Am. Chem. Soc.* **1980**, *102*, 5974.
[14] C. H. Behrens, S. Y. Ko, K. B. Sharpless, F. J. Walker, *J. Org. Chem.* **1985**, *50*, 5687.
[15] J. Turk, T. Stump, W. Conrad-Kessel, R. R. Seabold, B. A. Wolf, *Methods Enzymol.* **1990**, *187*, 175.
[16] R. Croteau, P. E. Kolattukudy, *Arch. Biochem. Biophys.* **1975**, *170*, 61.
[17] E. Blée, F. Schuber, *Biochem. J.* **1992**, *282*, 711.
[18] F. Kull, E. Ohlson, J. Z. Haeggström, *J. Biol. Chem.* **1999**, *274*, 34683.
[19] K. Mori, T. Otsuka, *Tetrahedron* **1983**, *39*, 3267.
[20] E. A. Couladouros, A. P. Mihou, *Tetrahedron Lett.* **1999**, *40*, 4861.
[21] S. A. Filppula, R. T. Sormunen, A. Hartig, W.-H. Kunau, J. K. Hiltunen, *J. Biol. Chem.* **1995**, *270*, 27453.
[22] J. D. Stewart, K. W. Reed, M. M. Kayser, *J. Chem. Soc., Perkin Trans. 1* **1996**, 755.
[23] L.-A. Garbe, R. Tressl, *Chem. Biodivers.* **2004**, *1*, 900.
[24] T. Haffner, R. Tressl, *J. Agric. Food Chem.* **1996**, *44*, 1218.

Received February 15, 2008