Metabolism of Deuterated Isomeric 6,7-Dihydroxydodecanoic Acids in *Saccharomyces cerevisiae* – Diastereo- and Enantioselective Formation and Characterization of 5-Hydroxydecano-4-lactone (=4,5-Dihydro-5-(1-hydroxyhexyl)furan-2(3*H*)-one) Isomers

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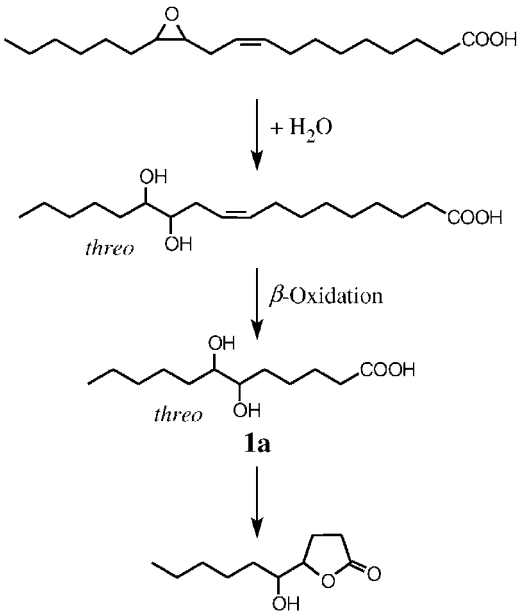
The chemical synthesis of deuterated isomeric 6,7-dihydroxydodecanoic acid methyl esters **1** and the subsequent metabolism of esters **1** and the corresponding acids **1a** in liquid cultures of the yeast *Saccharomyces cerevisiae* was investigated. Incubation experiments with (6*R*,7*R*)- or (6*S*,7*S*)-6,7-dihydroxy(6,7-$^2H_2$)dodecanoic acid methyl ester ((6*R*,7*R*)- or (6*S*,7*S*)-(6,7-$^2H_2$)-**1**, resp.) and (±)-*threo*- or (±)-*erythro*-6,7-dihydroxy(6,7-$^2H_2$)dodecanoic acid ((±)-*threo*- or (±)-*erythro*-(6,7-$^2H_2$)-**1a**, resp.) elucidated their metabolic pathway in yeast (*Tables 1–3*). The main products were isomeric ^2H-labeled 5-hydroxydecano-4-lactones **2**. The absolute configuration of the four isomeric lactones **2** was assigned by chemical synthesis *via Sharpless* asymmetric dihydroxylation and chiral gas chromatography (*Lipodex® E*). The enantiomers of *threo*-**2** were separated without derivatization on *Lipodex® E*; in contrast, the enantiomers of *erythro*-**2** could be separated only after transformation to their 5-*O*-(trifluoroacetyl) derivatives. Biotransformation of the methyl ester (6*R*,7*R*)-(6,7-$^2H_2$)-**1** led to (4*R*,5*R*)- and (4*S*,5*R*)-(2,5-$^2H_2$)-**2** (ratio *ca.* 4:1; *Table 2*). Estimation of the label content and position of (4*S*,5*R*)-(2,5-$^2H_2$)-**2** showed 95% label at C(5), 68% label at C(2), and no ^2H at C(4) (*Table 2*). Therefore, oxidation and subsequent reduction with inversion at C(4) of 4,5-dihydroxydecanoic acid and transfer of ^2H from C(4) to C(2) is postulated. The 5-hydroxydecano-4-lactones **2** are of biochemical importance: during the fermentation of *Streptomyces griseus*, (4*S*,5*R*)-**2**, known as L-factor, occurs temporarily before the antibiotic production, and (−)-muricatacin (=(4*R*,5*R*)-5-hydroxy-heptadecano-4-lactone), a homologue of (4*R*,5*R*)-**2**, is an anticancer agent.

Introduction. – The β-oxidation of fatty acids in the yeast *Saccharomyces cerevisiae* occurs inside the peroxisomes and is well-described [1][2]. In contrast, little is known about the metabolism of oxygenated fatty acids in yeast. Common enzymes introducing O-atoms into fatty acids are lipoxygenases, dioxygenases, and monooxygenases. In the mammalian cell, the cytochrome-P450-dependent monooxygenases transforming (*Z*)-unsaturated fatty acids into *cis*-epoxy acids are well-investigated, and further metabolic products are dihydroxy-substituted fatty acids [3]. Enzymes that utilize hydroperoxide as O-source are summarized in the terms 'epoxygenases' or 'peroxygenases' [4].

Epoxy fatty acids are chiral compounds. They can be found in mammalian and microbial cells but also in many plants, where they are involved in defense mechanisms [5]. Rice plants, *e.g.*, utilize linoleic acid (=(9*Z*,12*Z*)-octadeca-9,12-dienoic acid) to produce (+)-coronaric acid (=(9*R*,10*S*,12*Z*)-9,10-epoxyoctadec-12-enoic acid) [6], seeds from *Euphorbia sp.* contain optically active (+)-vernolic acid ((9*Z*,12*S*,13*R*)-12,13-epoxyoctadec-9-enoic acid) [7].

Incubation experiments with enantiomerically pure (+)-vernolic acid were performed with *Sporobolomyces odorus* [8], and (4*R*,5*R*)-5-hydroxydecano-4-lactone ((4*R*,5*R*)-**2**) was formed as the final product with 93.8% ee. The reference enantiomers of **2** were synthesized from amino acids and analyzed by achiral GC as derivatives obtained by reaction with (1*R*)-1-phenylethyl isocyanate. In analogous experiments, (±)-vernolic acid was converted to (4*R*,5*R*)-**2** with 74% ee., and 6,7-dihydroxydodecanoic acid (**1a**) was characterized as an intermediate [9]. The yeast *Saccharomyces cerevisiae* metabolized (±)-vernolic acid to mixtures of *threo*- and *erythro*-**2** (*Scheme 1*) [10].

Scheme 1. *Biotransformation of (±)-Vernolic Acid in Cultures of* Saccharomyces cerevisiae. The isomeric 5-hydroxydecano-4-lactones **2** were the main products in the culture broth.



The 5-hydroxydecano-4-lactones **2** are of biochemical importance. During the fermentation of *Streptomyces griseus*, the enantiomer (4*S*,5*R*)-**2** occurs temporarily before the antibiotic production and is known as L-factor in the literature [11][12]. Due to the biological activity of **2**, many contributions have been published concerning the chemical and chemoenzymatic synthesis of the four isomers of **2** [13–17]. *Sharpless* asymmetric dihydroxylation (*Sharpless* AD) [18] has been utilized to synthesize (+)-epimuricatacin and (−)-muricatacin (muricatacin = 5-hydroxyheptadecano-4-lactone) [19][20].

Investigations of *Blee* and *Schuber* demonstrated the hydrolysis of (±)-coronaric acid by purified soybean epoxide hydrolase to (9*R*,10*R*,12*Z*)-9,10-dihydroxyoctadec-12-enoic acid [21]. Therefore, the configuration of epoxy fatty acids cannot be deduced

by analysis of the corresponding dihydroxy fatty acids or further degradation products (*e.g.*, lactones). To get more insight into the biotransformation of 12,13-epoxyoleates to 5-hydroxydecano-4-lactones **2**, deuterated 6,7-dihydroxydodecanoic acids **1a** and their methyl esters **1** were used as precursors, *i.e.*, (±)-*threo*- and (±)-*erythro*-(6,7-$^2H_2$)-**1a** and (6*R*,7*R*)- and (6*S*,7*S*)-(6,7-$^2H_2$)-**1**.

Results. – 1. *Synthesis of Isomeric Methyl 6,7-dihydroxy(6,7-$^2H_2$)dodecanoates* **1**, *Corresponding Acids* **1a**, *and 5-Hydroxydecano-4-lactones* (**2**). The enantiomeric methyl esters (6*R*,7*R*)- and (6*S*,7*S*)-(6,7-$^2H_2$)-**1** were synthesized by *Sharpless* AD [18] of methyl (6*E*)-(6,7-$^2H_2$)dodec-6-enoate. The enantiomers (6*R*,7*R*)- and (6*S*,7*S*)-**1** could not be separated by chiral GC or HPLC. The *Sharpless* AD of methyl (*E*)-alkenoates of different C-chain lengths usually showed high stereoselectivity. Thus, AD of methyl (4*E*)-dec-4-enoate and lactonization of methyl 4,5-dihydroxydecanoate yielded (4*R*,5*R*)- and (4*S*,5*S*)-**2** with >98% ee.; the hydroxy-4-lactone enantiomers could be separated by chiral GC. 'AD-Mix α' and 'AD mix β' are reported to yield high amounts of *threo*-dihydroxy fatty acids, *e.g.*, methyl 9,10-dihydroxyoctadecanoate with >95% ee. [22]. Therefore, we assume a high ee. also for the *Sharpless* AD of methyl (6*E*)-(6,7-$^2H_2$)dodec-6-enoate yielding esters (6*S*,7*S*)- and (6*R*,7*R*)-(6,7-$^2H_2$)-**1**.

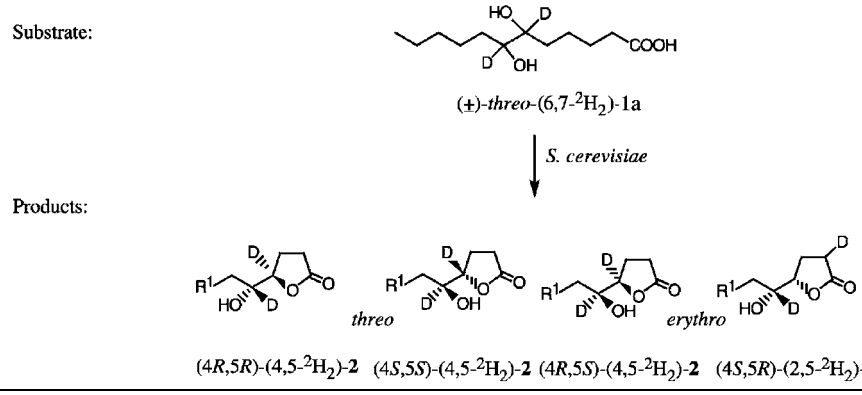
Racemic isomeric acids (±)-*threo*- and (±)-*erythro*-(6,7-$^2H_2$)-**1a** were synthesized by standard dihydroxylation procedures of (6*Z*)-(6,7-$^2H_2$)dodec-6-enoic acid (*threo:* 1. H_2O_2/HCOOH 2. NaOH; *erythro:* OsO_4). Deuteration of the (6*Z*)- and (6*E*)-double bond was >97% (^{1}H-NMR).

Diastereoisomers of **2** were analyzed on achiral (*DB-1*, *DB-Wax*) and enantiomers of **2** on chiral (*Lipodex® E*) GC phases. Enantiomers (4*R*,5*R*)- and (4*S*,5*S*)-**2** were synthesized by *Sharpless* AD [18] from methyl (4*E*)-dec-4-enoate. Acid-catalyzed lactonization of methyl 4,5-dihydroxydecanoates gave **2** with high ee. (>98%; chiral GC). *Dess-Martin* oxidation of (4*R*,5*R*)-**2** led to (4*R*)-5-oxodecano-4-lactone, and a $NaBH_4$ reduction yielded an analytic reference, *i.e.*, (4*R*,5*S*)-**2**. An analogous experiment with (4*S*,5*S*)-**2** generated (4*S*,5*R*)-**2**. The diastereoisomers of **2** were separated on *DB-1* after formation of their 5-*O*-(trimethylsilyl)ethers in the order *erythro*-**2** derivative > *threo*-**2** derivative. On chiral GC *Lipodex® E* phases, the free isomers of **2** were eluted in the order (4*S*,5*S*)-**2** > (4*R*,5*R*)-**2** > (±)-*erythro*-**2** (no resolution of the *erythro*-enantiomers), and their 5-*O*-(trifluoroacetyl) derivatives in the order (4*R*,5*S*)-**2** > (4*S*,5*R*)-**2** > (±)-*threo*-**2** (no resolution of *threo*-enantiomers) (see below, *Fig. 4*).

2. *Incubation Experiments.* The results of the incubations of racemic acid (±)-*threo*-(6,7-$^2H_2$)-**1a** and enantiomeric esters (6*R*,7*R*)- and (6*S*,7*S*)-(6,7-$^2H_2$)-**1** with *S. cerevisiae* are summarized in *Tables 1–3*. During the course of the fermentation of the racemic acids **1a** and enantiomeric methyl esters **1**, the substrates were metabolized to deuterated hydroxy-oxo acids and 5-hydroxydecano-4-lactones as major products. One cycle of β-oxidation of **1** or **1a** would lead to 4,5-dihydroxydecanoic acids, which were not detectable in the fermentation broth. β-Oxidation to 2,3-dihydroxyoctanoate was not observed.

In incubation experiments of (±)-*threo*-(6,7-$^2H_2$)-**1a**, four isomeric 5-hydroxy-4-lactones **2** were characterized as doubly labeled isotopomers with a yield of 3–4% (*Table 1*). The ratio *threo*-**2**/*erythro*-**2** was analyzed as 92:8. Chiral analysis of the

Table 1. *Total Amounts* [ppm], *Relative Ratios, and Distribution of the* ^2H*-Label of Isomeric-5-Hydroxy(*^2H_x*)-decano-4-lactones* **2** *after Incubation of 150 ppm of* (±)-threo-*6,7-Dihydroxy(6,7-*$^2H_2$*)dodecanoic Acid* **1a** *in Liquid Cultures of* Saccharomyces cerevisiae. $R^1 = Me(CH_2)_3$.



	(4*R*,5*R*)-(4,5-$^2H_2$)-**2**	(4*S*,5*S*)-(4,5-$^2H_2$)-**2**	(4*R*,5*S*)-(4,5-$^2H_2$)-**2**	(4*S*,5*R*)-(2,5-$^2H_2$)-**2**
Amount	2.85 ppm	0.76 ppm	0.02 ppm	0.28 ppm
Ratio	72.9%	19.4%	0.5%	7.2%
^2H-Label at C(2)	0%	n.d.	n.d.	86%
^2H-Label at C(4)	95%	95%	n.d.	0%
^2H-Label at C(5)	95%	95%	n.d.	95%

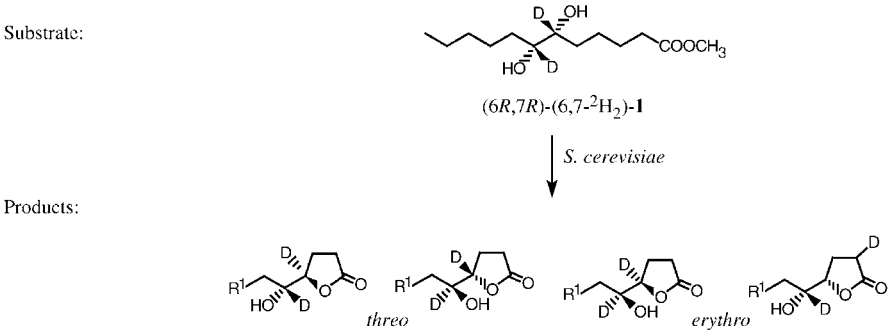
products indicated (4*R*,5*R*)-**2** as major metabolite (72.9%) besides 19.4% of (4*S*,5*S*)-**2**, 7.2% of (4*S*,5*R*)-**2**, and only 0.5% of (4*R*,5*S*)-**2**.

The main products formed from methyl ester (6*R*,7*R*)-(6,7-$^2H_2$)-**1** were (4*R*,5*R*)-**2** and (4*S*,5*R*)-**2**. They accumulated in a ratio of 78.5:21.5 with a total yield of 44% (*Table 2*). The separation of the isomers of **2** showed unequivocally the inversion of the configuration at C(4) from (4*R*) to (4*S*).

This inversion might be explained by oxidation to a keto acid followed by a reduction step. We identified isomeric α-ketols, *i.e.*, methyl 6-hydroxy-7-oxododecanoate and methyl 7-hydroxy-6-oxododecanoate (see below, *Fig. 1*), during the metabolism of **1** and **1a** by GC/MS as major products. They had a maximum in concentration after 168 h, and their amount decreased until the end of fermentation. But no reduction of the α-ketols into *erythro*-**1** and no ^2H-depletion at position C(6) or C(7) of the substrate **1** or **1a** was observed by GC/MS. The identification of a doubly labeled (4*S*,5*R*)-**2** is not in agreement with an oxidation/keto acid reduction step.

3. *Analysis of Isotopomers.* The ^2H-content of the substrates **1** and **1a** (methylated, silylated) was analyzed during the incubation period by GC/EI-MS by means of the characteristic fragments *m*/*z* 173/174 and 217/218 (see *Fig. 1*) [23]. No significant ^2H-depletion was observed for (6,7-$^2H_2$)-**1** and -**1a**. The label content of the side chain of **2** (position C(5)) was measured by analyzing *m*/*z* 173/174 ($^1H_1/^2H_1$) (α-cleavage) comparable to silylated dihydroxy fatty acid esters. The ^2H-content of the lactone ring was quantified by *m*/*z* 158/159 ($^1H_1/^2H_1$). The Me_3Si-group migration in 5-*O*-(trimethylsilyl)derivatives to the lactone moiety is revealed by *Fig. 2*. The Me_3Si-

Table 2. *Total Amounts* [ppm], *Relative Ratios, and Distribution of the* ^2H*-Label of Isomeric-5-Hydroxy(*^2H_x*)-decano-4-lactones* **2** *after Incubation of 150 ppm of (6*R,7R*)-6,7-Dihydroxy(6,7-*$^2H_2$*)dodecanoic Acid Methyl Ester* **1** *in Liquid Cultures of* Saccharomyces cerevisiae. $R^1 = Me(CH_2)_3$.



	(4*R*,5*R*)-(4,5-$^2H_2$)-**2**	(4*S*,5*S*)-(4,5-$^2H_2$)-**2**	(4*R*,5*S*)-(4,5-$^2H_2$)-**2**	(4*S*,5*R*)-(2,5-$^2H_2$)-**2**
Amount	40.6 ppm	0 ppm	0 ppm	11.1 ppm
Ratio	78.5%	0%	0%	21.5%
^2H-Label at C(2)	0%	–	–	68%
^2H-Label at C(4)	96%	–	–	0%
^2H-Label at C(5)	97%	–	–	98%

group rearrangement from C(5) to the lactone ring under EI-MS conditions could be established by several experiments. The nonderivatized hydroxy-lactones **2** showed a H-rearrangement from OH−C(5) to the γ-lactone moiety (m/z 85 + 1). This H-transfer was secured by O^{2}H−C(5) shifts in ^2H_2O (data not shown). In contrast, 5-*O*-(trifluoroacetyl) derivatives of **2** did not rearrange and showed α-cleavage, as established by the results of *Fig. 3*. These EI-MS results were confirmed by measurements with unlabeled references.

In the case of the incubation of ester (6*R*,7*R*)-(6,7-$^2H_2$)-**1**, we identified doubly labeled (4*R*,5*R*)-**2** and unexpectedly doubly labeled (4*S*,5*R*)-**2**. The inversion at the chiral center C(4) by the oxidation/keto reduction pathways with intermediate α-ketols (*Fig. 1*) would be a suitable explanation. But, instead of total ^2H-loss, we detected a 68% lactone-ring label and a 98%-labeled side chain (C(5)) for (4*S*,5*R*)-**2** in this experiment (see above, *Table 2*). The amounts of (4*R*,5*R*)-**2** and (4*S*,5*R*)-**2** increased during fermentation in a distinct ratio of 4 : 1 and showed concentration maxima at the end of the fermentation. The initial ^2H-contents of the side chain (C(5)) were retained in both lactones. The lactone-ring label of (4*S*,5*R*)-**2** was decreased from initially 86% to 68%. In contrast to that, the ring label content of (4*R*,5*R*)-**2** did not change and was 96%.

^2H-Depletion at C(4) and C(3) is not favored but plausible at the acidic position C(2). This ^2H-exchange could be demonstrated in model experiments with (2,2-$^2H_2$)decano-4-lactone. Therefore, we postulate a (2,5-$^2H_2$)-labeled (4*S*,5*R*)-**2**, which is in agreement with the postulated metabolism (see below, *Scheme 2*).

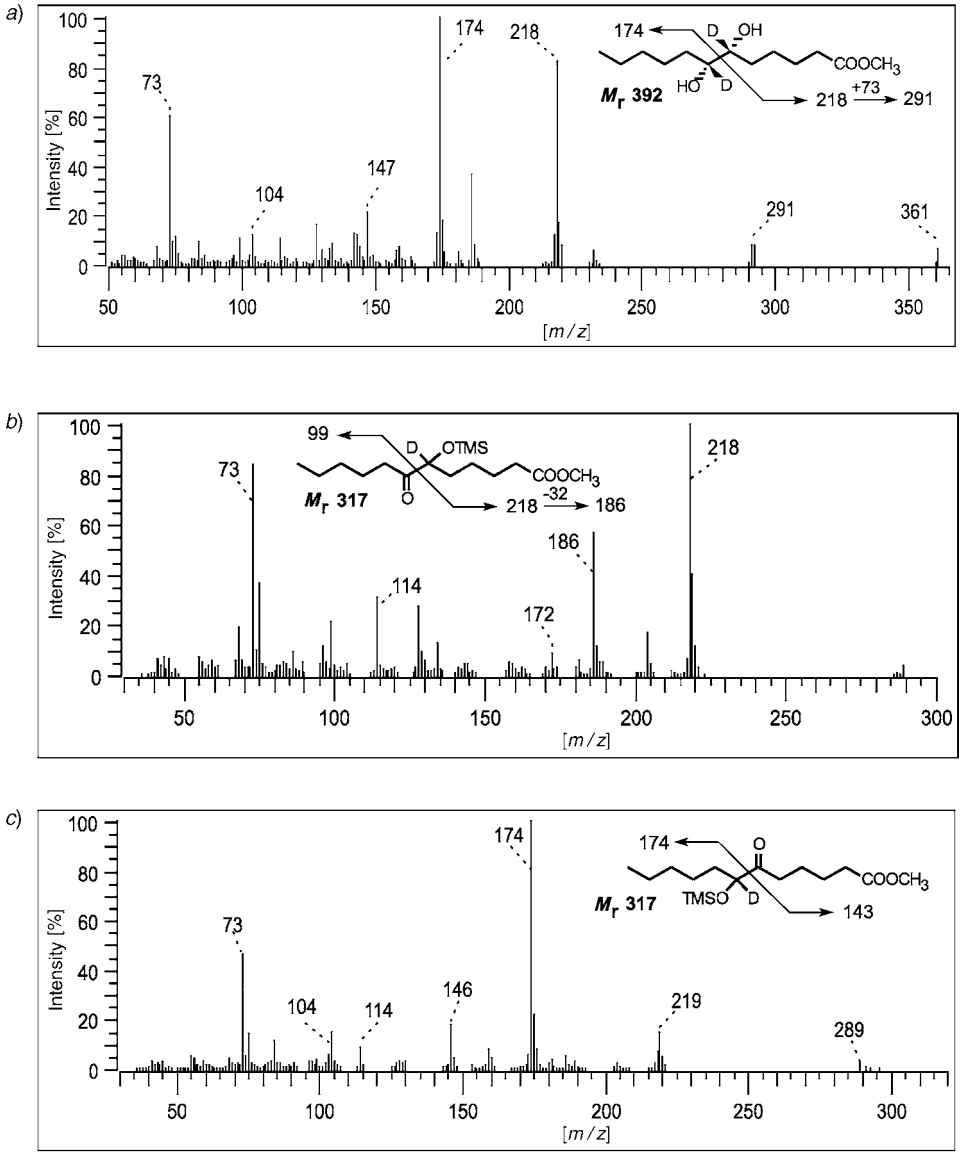


Fig. 1. *Low-Resolution EI-MS of* a) *methyl ester (6*R,7R*)*-(6,7-$^2H_2$)-**1** (synthesized, Me_3Si ether), b) *methyl 6-hydroxy-7-oxo(6-$^2H_1$)dodecanoate* (postulated), *and* c) *methyl 7-hydroxy-6-oxo(7-$^2H_1$)dodecanoate* (postulated). Both α-ketols were isolated from culture broth of incubation experiment of **1** and **1a** in *S. cerevisiae*. TMS = Me_3Si.

Incubation experiments of methyl ester (6*S*,7*S*)-(6,7-$^2H_2$)-**1** showed the degradation into four hydroxy-4-lactones **2** with 6.8% yield (*Table 3*). (4*S*,5*S*)-(4,5-$^2H_2$)-**2** was characterized as the main product (92.6%). Inversion to *erythro*-**2** comparable to that

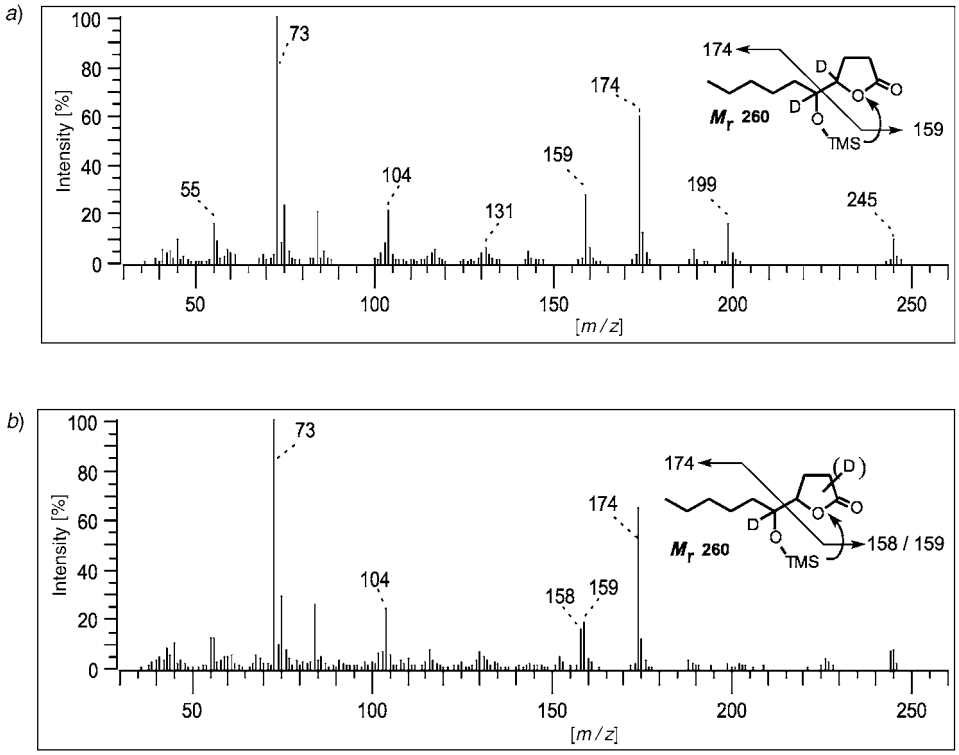


Fig. 2. *Low-Resolution EI-MS of* a) *(4,5-$^2H_2$)*-**2** (Me_3Si ether) *and* b) *(^2H_x)*-**2** (Me_3Si ether) *with significant lactone-ring ^2H-depletion, isolated from cultures of* S. cerevisiae *after incubation of methyl ester (6*R*,7*R*)-(6,7-$^2H_2$)*-**1**. TMS = Me_3Si.

described in the experiment with methyl ester (6*R*,7*R*)-(6,7-^{2}H)-**1** was not observed. The minor amounts of (4*R*,5*R*)-**2** and (4*S*,5*R*)-**2** might be explained by a small amount of (6*R*,7*R*)-(6,7-$^2H_2$)-**1** present in the incubation substrate.

In incubation experiments of acid (±)-*erythro*-(6,7-$^2H_2$)-**1a**, four isomeric 5-hydroxy-4-lactones **2** were characterized as doubly labeled isotopomers with a yield of 3.1%. The ratio *erythro*-**2**/*threo*-**2** was 95 : 5. Chiral analysis of the products indicated the presence of 65.3% of (4*S*,5*R*)-(4,5-$^2H_2$)-**2**, 29.2% of (4*R*,5*S*)-(4,5-$^2H_2$)-**2**, and minor amounts of *threo*-**2** (*Table 4*). In contrast to the case of the (±)-*threo*-**1** incubations, the (4*S*)-isomer was predominantly lactonized.

Discussion. – The biotransformations of monohydroxy fatty acids [24][25] have been well-investigated, whereas the degradation of dihydroxy fatty acids are mostly unknown. The 6,7-dihydroxydodecanoic acids **1a**, which are formed as intermediates during the vernolic acid metabolism, are transformed by one cycle of β-oxidation into 4,5-dihydroxydecanoyl-CoA, which is immediately lactonized to **2**. A further degradation into 2,3-dihydroxyoctanoyl-CoA, hexanoyl-CoA, and 2-hydroxyacetyl-CoA has not been observed [26]. In addition, the substrates **1** and **1a** are oxidized to the

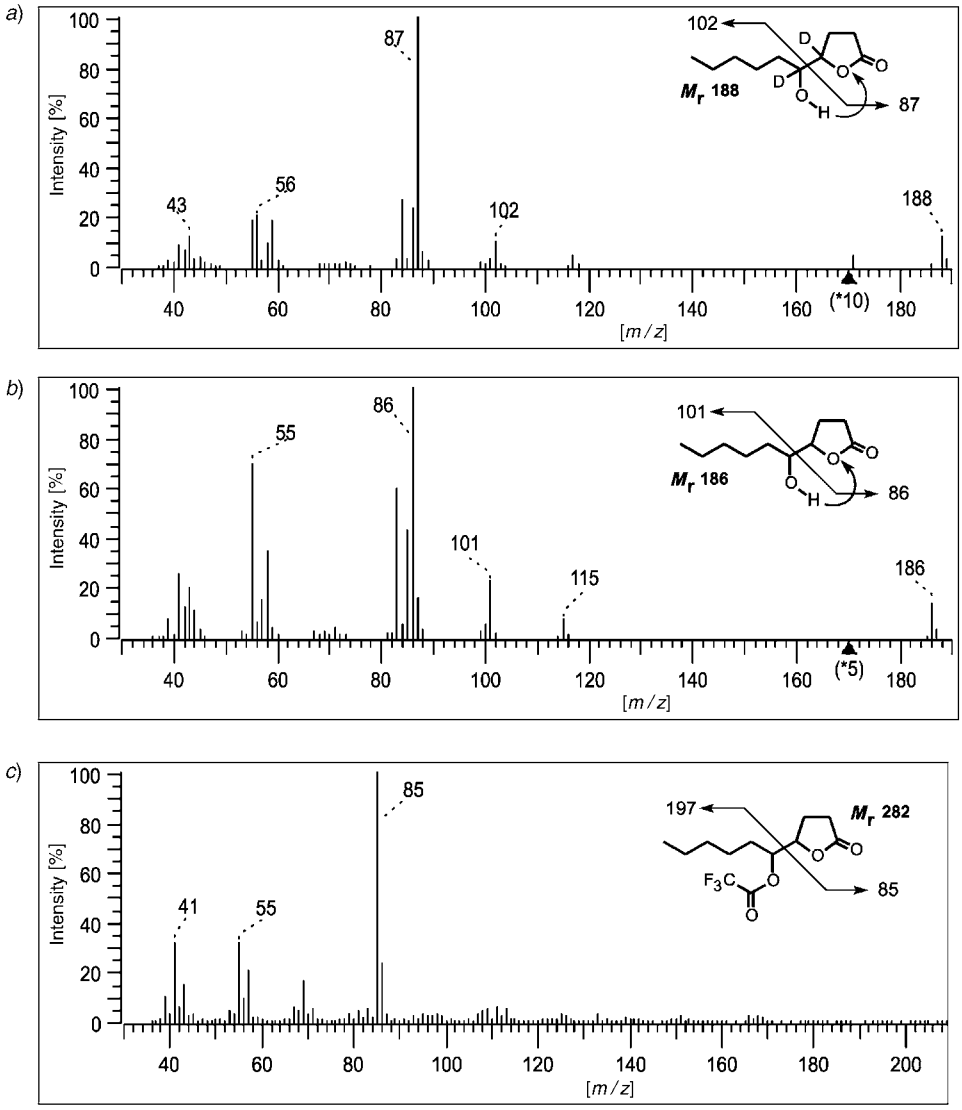


Fig. 3. *Low-Resolution EI-MS of* a) *(4,5-$^2H_2$)*-**2** *isolated from cultures of* S. cerevisiae *after incubation of methylester (6*R,7R*)-(6,7-$^2H_2$)*-**1**, b) *synthesized* **2**, *and* c) *5*-O-*(trifluoroacetyl) derivative of synthesized* **2**

corresponding α-ketols in oscillating concentrations which disappear at the end of the fermentation. The (±)-*threo*- and (±)-*erythro*-6,7-dihydroxydodecanoic acids **1a** were efficiently metabolized by *S. cerevisiae*, and only 3–4% was converted to the hydroxylactones **2**. In corresponding incubations of the methyl esters **1** of the *threo*-enantiomers, 10–40% of the substrates were transformed into hydroxylactones, and high amounts of **1** were not metabolized. The racemic dihydroxy acid *threo*-**1a** formed *threo*-**2**/*erythro*-**2** in a ratio of 92:8 with (4*R*,5*R*)-**2** as the main product. The

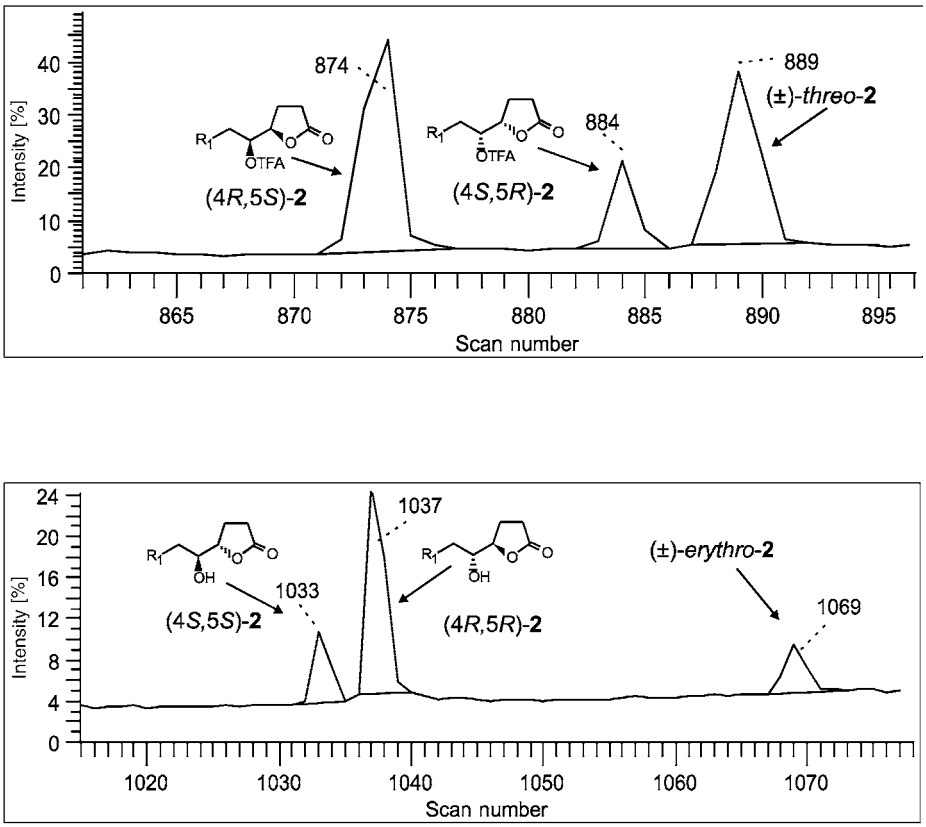


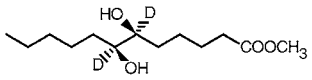
Fig. 4. *Chiral gas chromatographic resolution (Lipodex® E) of isomeric* **2** *with and without trifluoroacetic acid (TFA) derivatization*

metabolism of the corresponding acid (±)-*erythro*-**1a** showed a comparable pattern, with an *erythro*-**2**/*threo*-**2** ratio of 95:5 and (4*S*,5*R*)-**2** as the major product. Lactonization of *threo*-4,5-dihydroxy acids is preferred with (4*R*)-configuration, whereas from *erythro*-4,5-dihydroxy acids, the (4*S*)-configured center was predominantly esterified. The hydroxy-4-lactones and the substrates possessed comparable ^{2}H-contents.

The result of the (6*R*,7*R*)-(6,7-$^2H_2$)-**1** metabolism was of particular interest because the presence of 25% of (4*S*,5*R*)-(2,5-$^2H_2$)-**2** was observed in the fermentation broth. During fermentation, the ^{2}H-content of the lactone ring of (4*S*,5*R*)-(2,5-$^2H_2$)-**2** was decreasing from 86% to 68%, whereas the ^{2}H-content of (4*R*,5*R*)-(4,5-$^2H_2$)-**2** did not change. The enantiomer (4*S*,5*R*)-**2** is the bioactive L-factor in *Streptomyces griseus* fermentation. Therefore, we postulate a degradation pathway of methyl ester (6*R*,7*R*)-(6,7-$^2H_2$)-**1** according to *Scheme 2*, wherein the transfer of ^{2}H is catalyzed by an enone reductase. Isomeric α-ketols were the products with the highest concentration in all experiments, and their amounts continuously oscillated during fermentation. But direct

Table 3. *Total Amounts* [ppm], *Relative Ratios, and Distribution of the* ^2H*-Label of Isomeric-5-Hydroxy(*^2H_x*)-decano-4-lactones* **2** *after Incubation of 150 ppm of (6S,7S)-6,7-Dihydroxy(6,7-*$^2H_2$*)dodecanoic Acid Methyl Ester* **1** *in Liquid Cultures of* Saccharomyces cerevisiae. $R^1 = Me(CH_2)_3$.

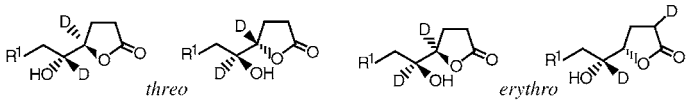
Substrate:



(6*S*,7*S*)-(6,7-$^2H_2$)-**1**

S. cerevisiae

Products:

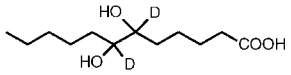


threo *erythro*

	(4*R*,5*R*)-(4,5-$^2H_2$)-**2**	(4*S*,5*S*)-(4,5-$^2H_2$)-**2**	(4*R*,5*S*)-(4,5-$^2H_2$)-**2**	(4*S*,5*R*)-(2,5-$^2H_2$)-**2**
Amount	0.3 ppm	7.4 ppm	0.06 ppm	0.23 ppm
Ratio	3.7%	92.6%	0.8%	2.9%
^2H-Label at C(2)	0%	n.d.	n.d.	79%
^2H-Label at C(4)	95%	95%	n.d.	0%
^2H-Label at C(5)	97%	97%	n.d.	94%

Table 4. *Total Amounts* [ppm], *Relative Ratios, and Distribution of the* ^2H*-Label of Isomeric-5-Hydroxy(*^2H_x*)-decano-4-lactones* **2** *after Incubation of 150 ppm of (±)-*erythro*-6,7-Dihydroxy(6,7-*$^2H_2$*)dodecanoic Acid* **1a** *in Liquid Cultures of* Saccharomyces cerevisiae. $R^1 = Me(CH_2)_3$.

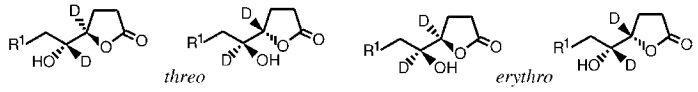
Substrate:



(±)-*erythro*-(6,7-$^2H_2$)-**1a**

S. cerevisiae

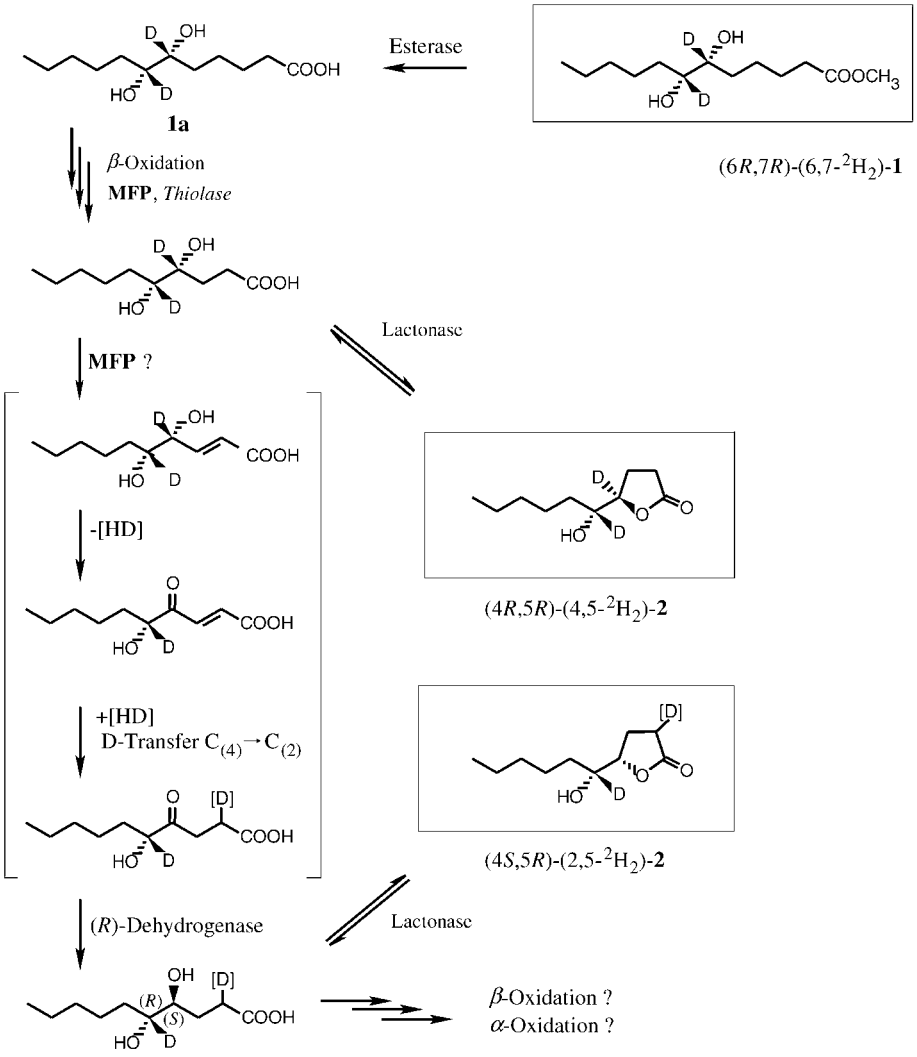
Products:



threo *erythro*

	(4*R*,5*R*)-(4,5-$^2H_2$)-**2**	(4*S*,5*S*)-(4,5-$^2H_2$)-**2**	(4*R*,5*S*)-(4,5-$^2H_2$)-**2**	(4*S*,5*R*)-(4,5-$^2H_2$)-**2**
Amount	0.13 ppm	0.07 ppm	1.05 ppm	2.35 ppm
Ratio	3.6%	1.9%	29.2%	65.3%
^2H-Label at C(2)	75%	n.d.	0%	0%
^2H-Label at C(4)	0%	n.d.	97%	93%
^2H-Label at C(5)	92%	n.d.	96%	96%

Scheme 2. *Postulated Metabolism of Methyl Ester (6*R,7R*)-(6,7-*$^2H_2$*)-***1*** *in Liquid Cultures of* Saccharomyces cerevisiae. MFP = Multifunctional protein.



reduction of α-ketols to ^{2}H-depleted *erythro*-**1** or *threo*-**1** or -**1a** could not be detected. Therefore, a *Baeyer–Villiger*-type oxidation of the α-ketols might be involved in the pathway.

Experimental Part

1. *General*. LC = liquid chromatography. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AMX-500* spectrometer (Karlsruhe, Germany); chemical shifts δ in ppm rel. to $SiMe_4$ (= 0 ppm) as external standard, *J* in Hz. GC/MS:

fused silica *DB-1* capillary column (poly(dimethylsiloxane)), 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 & Nagel*, Düren, Germany), 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-ionization *Varian Mat-8230* mass spectrometer, ionization energy 70 eV; *m*/*z* (intensity in %).

2. *(±)*-threo- *and (±)*-erythro-*6,7-Dihydroxy(6,7-$^2H_2$)dodecanoic Acids* **1a** *and Methyl (6*R*,7*R*)- and Methyl (6*S*,7*S*)-6,7-Dihydroxy(6,7-$^2H_2$)dodecanoates* **1**.

2.1. *Dodec-6-ynenitrile*. At 0° under N_2, 2.5M BuLi in hexane (22 ml) was added dropwise to hept-1-yne (4.4 g, 45 mmol) in abs. THF (40 ml) and stirred for 2 h. Then 1,4-dibromobutane (19.5 g, 90 mmol) in abs. DMPU (= 3,4,5,6-tetrahydro-1,3-dimethylpyrimidin-2(1*H*)-one; 100 ml) was rapidly added at 0°. The mixture was stirred at 20° for 24 h, refluxed for 2 h, cooled to 10°, mixed with ice/H_2O (60 ml), and extracted with petroleum ether (3 × 50 ml). The combined extracts were washed with brine (50 ml), dried (Na_2SO_4), and evaporated: crude *1-bromoundec-5-yne* (14.0 g). GC/MS: 151 (3, $[M-Br]^+$), 134 (2), 121 (2), 109 (25), 95 (10), 93 (15), 91 (15), 82 (10), 81 (60), 79 (30), 77 (25), 69 (15), 68 (15), 67 (90), 65 (15), 55 (45), 54 (55), 53 (35), 52 (20), 51 (15), 43 (15), 42 (10), 41 (100), 40 (10), 39 (60).

At 80° under N_2, the crude 1-bromoundec-5-yne was added dropwise to NaCN (8.2 g, 160 mmol) in abs. DMSO (80 ml). The mixture was stirred at 80° for 2 h and at 20° for 24 h. After addition of dist. H_2O (100 ml), the mixture was extracted with Et_2O (2 × 50 ml), the combined Et_2O phase washed with H_2O (100 ml), 6M HCl (30 ml), H_2O (2 × 50 ml), and brine (50 ml) and evaporated, and the crude product purified by LC silica gel (50 g), petroleum ether/AcOEt 18:2 (500 ml): 6.2 g (77% based on hept-1-yne) of *dodec-6-ynenitrile*. GC/MS: 176 (3, $[M-1]^+$), 162 (5), 148 (10), 134 (60), 120 (30), 107 (25), 106 (25), 95 (25), 94 (15), 93 (35), 91 (20), 83 (5), 82 (10), 81 (50), 80 (25), 79 (44), 77 (30), 68 (10), 67 (75), 66 (10), 65 (21), 56 (10), 55 (35), 54 (30), 53 (30), 52 (25), 51 (24), 50 (10), 43 (15), 42 (17), 41 (100), 40 (20), 39 (60).

2.2. *Dodec-6-ynoic Acid*. At 20° under N_2, Na_2O_2 (6.2 g, 80 mmol) was added to dodec-6-ynenitrile (6.2 g, 35 mmol) in abs. DMSO (40 ml). The mixture was stirred for 24 h at 20° and, after addition of H_2O (80 ml), 2 h at 80°. The mixture was cooled to 20°, acidified with 6M HCl and extracted with Et_2O (2 × 50 ml). The combined Et_2O extract was washed with brine (50 ml), dried (Na_2SO_4), and evaporated, and the crude acid purified by LC (silica-gel (50 g), petroleum ether/AcOEt 18:2 (100 ml), petroleum ether/AcOEt/AcOH 18:2:0.2 (200 ml) and petroleum ether/AcOEt/AcOH 15:5:0.2 (400 ml)): 4.6 g (67%) of *dodec-6-ynoic acid*. GC/MS (corresponding methyl ester): 210 (2, M^+), 178 (5, $[M-MeOH]^+$), 161 (2), 154 (25), 136 (15), 135 (8), 122 (15), 121 (17), 107 (15), 96 (8), 95 (30), 94 (55), 93 (40), 91 (30), 82 (18), 81 (56), 80 (100), 79 (90), 78 (20), 77 (30), 74 (25), 69 (8), 68 (15), 67 (64), 66 (15), 65 (22), 59 (39), 57 (12), 55 (48), 54 (28), 53 (26), 52 (24), 51 (20), 43 (30), 42 (18), 41 (88), 40 (15), 39 (45).

2.3. *(6*Z*)-(6,7-$^2H_2$)Dodec-6-enoic Acid*. At 0°, 5% Pd/$BaSO_4$ (60 mg) was stirred in MeOH (80 ml). Quinoline (0.75 ml) was added, and the mixture was evacuated and aerated with $^2H_2$ gas (*Messer-Griesheim*, Germany, 2.7). Addition of dodec-6-ynoic acid (6.7 mmol) in MeOH (5 ml) started the consumption of the theoretical volume of 150 ml of $^2H_2$. The catalyst was filtered off and the filtrate diluted with Et_2O (50 ml) and H_2O (50 ml) and acidified with 2M HCl. The Et_2O phase was washed (brine), dried (Na_2SO_4), evaporated and the crude acid purified by LC (silica gel (50 g), petroleum ether/AcOEt/AcOH 18:2:0.2 (400 ml)): 1.2 g (89%) of *(6*Z*)-6,7-$^2H_2$)dodec-6-enoic acid*. GC/MS (corresponding methyl ester): 214 (22, M^+), 183 (30), 182 (40), 165 (15), 153 (8), 141 (10), 140 (61), 139 (20), 138 (13), 126 (10), 125 (22), 124 (12), 111 (25), 110 (25), 99 (45), 98 (72), 97 (85), 96 (35), 88 (20), 87 (46), 86 (15), 85 (57), 84 (85), 83 (70), 82 (40), 81 (25), 75 (25), 74 (100), 71 (32), 70 (52), 69 (70), 68 (35), 59 (40), 57 (55), 56 (88), 55 (76), 54 (18), 44 (15), 43 (62), 40 (52), 41 (69), 40 (15), 39 (20).

2.4. *(6*E*)-(6,7-$^2H_2$)Dodec-6-enoic Acid*. At 0° under N_2, $LiAl^2H_4$ (1.88 g, 44.6 mmol; >98% ^2H atom) was suspended in abs. diethylene glycol dimethyl ether (= diglyme; 50 ml), and dodec-6-ynoic acid (1.45 g, 7.4 mmol) in diglyme (5 ml) was added very slowly and stirred for 15 min. The mixture was heated to 150° for a minimum of 72 h, hydrolyzed at 10° with ^2H_2O (15 ml; >98% ^2H atom; *Campro Scientific*, The Netherlands), heated to 60° for 30 min, acidified with 2M HCl, and extracted with petroleum ether (3 ×). The combined org. phase was washed with H_2O (50 ml) and brine (50 ml), dried (Na_2SO_4), and evaporated, and the crude alcohol submitted to LC (silica gel (50 g), petroleum ether/AcOEt 18:2 (500 ml); 0.93 g (67%) of *(6*E*)-(6,7-$^2H_2$)dodec-6-en-1-ol*.

At 20°, pyridinium chlorochromate (2.1 g, 10 mmol) and anh. NaOAc (80 mg) were dissolved in CH_2Cl_2 (20 ml). Then (6*E*)-(6,7-$^2H_2$)dodec-6-en-1-ol (0.93 g, 5 mmol) was added and stirred for 3 h. After addition of petroleum ether (20 ml), the mixture was filtered through a silica-gel (10 g) column, which was washed with CH_2Cl_2 (50 ml). Evaporation gave 0.83 g (90%) of *(6*E*)-(6,7-$^2H_2$)dodec-6-enal*. GC/MS: 166 (15, $[M-18]^+$),

140 (8), 123 (10), 114 (8), 113 (7), 112 (9), 111 (5), 110 (4), 109 (6), 99 (25), 98 (30), 97 (24), 96 (15), 95 (15), 85 (15), 84 (24), 83 (40), 82 (25), 81 (25), 80 (20), 71 (25), 70 (48), 69 (42), 68 (26), 67 (20), 57 (55), 56 (85), 55 (70), 54 (15), 44 (20), 43 (78), 42 (75), 41 (100), 40 (27), 39 (35).

At 20°, ^{t}BuOH (8 ml) and 2-methylbut-2-ene (2 ml) were mixed with a freshly prepared soln. of $NaClO_2$ (0.33 g, 3.7 mmol) and NaH_2PO_4 (0.38 g, 3.1 mmol) in H_2O (4 ml). Crude (6*E*)-(6,7-$^2H_2$)dodec-6-enal (0.83 g, 4.5 mmol) was added and stirred for 18 h. After evaporation, the residue was dissolved in H_2O (20 ml), acidified with 2M HCl (20 ml), and extracted with Et_2O (3 × 50 ml). The combined Et_2O phases were washed (sat. $Na_2S_2O_3$ soln. (20 ml) and brine (50 ml)) and the crude acid was purified by LC (silica gel (50 g), petroleum ether/AcOEt/AcOH 18:2:0.2 (400 ml)): 0.7 g (47% based on dodec-6-ynoic acid) of *(6*E*)-(6,7-$^2H_2$)dodec-6-enoic acid*. ^{1}H-NMR (corresponding methyl ester, $CDCl_3$): 0.75 (*t*, *J* = 7, Me); 1.1–1.3 (*m*, 8 H, CH_2); 1.49 (*quint.*, *J* = 7, C*H*$_2$CH$_2$COOMe); 1.85 (*m*, C*H*$_2$CH=CHC*H*$_2$); 2.19 (*t*, *J* = 7, C*H*$_2$COOMe); 3.53 (*s*, COO*Me*); 0.07 H for signal of H–C(6) and H–C(7), *i.e.*, labeling 97%. ^{13}C-NMR ($CDCl_3$): 13.97 (C(12)); 22.46 (C(11)); 24.34; 28.96; 29.17; 31.32; 31.92; 32.30; 33.86; 51.33 (MeO); 128.99 (*t*, *J* = 22, C(7)); 130.42 (*t*, *J* = 22, C(6)); 174.14 (C(1)). GC/MS (corresponding methyl ester): 214 (11, M^+), 182 (19), 165 (15), 164 (15), 153 (10), 140 (35), 125 (25), 112 (20), 111 (20), 99 (30), 98 (40), 97 (60), 96 (28), 87 (35), 86 (15), 85 (40), 84 (55), 83 (50), 82 (30), 81 (27), 75 (25), 74 (100), 71 (30), 70 (40), 69 (55), 68 (29), 67 (18), 59 (60), 57 (55), 56 (80), 55 (75), 54 (25), 44 (15), 43 (80), 42 (77), 41 (95), 40 (30), 39 (35), 31 (15).

2.5. *(±)*-threo-*Dihydroxy Acids* threo-**1a**: *General Procedure.* At 0°, H_2O_2 (10 ml; 30% in H_2O), conc. formic acid (50 ml), and (*Z*)-alkenoic acid (4 mmol) were stirred for 5 min, then for 1 h at 40° and overnight at 20°. The mixture was acidified with 6M HCl and extracted with Et_2O (3 ×). The combined extract was washed with brine (50 ml), dried (Na_2SO_4), and evaporated. The crude product was hydrolyzed with 1M NaOH (40 ml) at 100° for 1 h. Acidification of the mixture (6M HCl), addition of solid NaCl (5 g), and extraction with Et_2O (3 ×), re-extraction of the combined Et_2O phases with brine (50 ml), drying (Na_2SO_4), and evaporation afforded the crude dihydroxy fatty acid, which was purified by LC (silica gel (50 g), petroleum ether/AcOEt 18:2 (100 ml), petroleum ether/AcOEt/AcOH 18:2:0.2 (200 ml), and petroleum ether/AcOEt/AcOH 15:5:0.2 (400 ml)): 2.4 mmol (60%) of the *threo*-dihydroxy acid *threo*-**1a**, containing *ca.* 2% of the *erythro*-diastereoisomer (by TLC and GC).

(±)-threo-*6,7-Dihydroxy(6,7-$^2H_2$)dodecanoic Acid* ((±)-*threo*-(6,7-$^2H_2$)-**1a**): ^{1}H-NMR ($CDCl_3$): 0.70 (*t*, *J* = 7, Me); 1.0–1.5 (*m*, 14 H, CH_2); 2.13 (*t*, *J* = 7, C*H*$_2$COOH); 0.06 H for signal of H–C(6) and H–C(7), *i.e.*, labeling 97%. ^{13}C-NMR ($CDCl_3$): 13.60 (C(12)); 22.28 (C(11)); 24.56; 25.38; 30.47; 31.02; 31.60; 32.80; 33.71; 73.59, 73.75, 73.89, 74.06 (*m*, C(6), C(7)); 176.44 (C(1)).

2.6. *(±)*-erythro-*Dihydroxy Acid* erythro-**1a**: *General Procedure.* (*Z*)-Alkenoic acid in ^{t}BuOH was oxidized with stoichiometric amounts of OsO_4. The mixture was worked up as described in *Exper. 2.5*. (±)-*erythro*-dihydroxy acid *erythro*-**1a** containing *ca.* 4% (GC) of the *threo*-diastereoisomer.

(±)-erythro-*6,7-Dihydroxy(6,7-$^2H_2$)-dodecanoic Acid:* ((±)-*erythro*-(6,7-$^2H_2$)-**1a**): ^{1}H-NMR ($CDCl_3$): 0.70 (*t*, *J* = 7, Me); 1.0–1.5 (*m*, 14 H, CH_2); 2.13 (*t*, *J* = 7, C*H*$_2$COOH); 0.06 H for signal of H–C(6) and H–C(7), *i.e.*, labeling 97%. ^{13}C-NMR ($CDCl_3$): 13.58 (C(12)); 22.28 (C(11)); 24.56; 25.16; 25.35; 31.59; 32.50; 32.79; 33.72; 72.97, 73.25, 73.41, 73.61 (*m*, C(6), C(7)); 176.42 (C(1)).

2.7. *Methyl (4*E*)-Dec-4-enoate* was synthesized from commercially available (4*E*)-dec-4-enal by $NaClO_2$ oxidation (see *Exper. 2.4*) and MeOH/HCl methylation.

2.8. *Asymmetric Dihydroxylation* (AD): *Formation of Dihydroxyalkanoic Acid Methyl Esters* **1**. AD was performed according to the *Sharpless AD* method [18]. Methyl (*E*)-alkenoates yielded (*S,S*)-dihydroxy fatty acid methyl esters with 'AD-Mix α' and (*R,R*)-dihydroxy fatty acid methyl esters with 'AD-Mix β'. In a typical procedure, ^{t}BuOH (5 ml), H_2O (5 ml), 'AD-Mix α' or 'AD Mix β' (1.4 g), and methansulfonamide ($MeSO_2NH_2$; 95 mg, 1 mmol) were mixed at 20° and chilled to 0°. Methyl (*E*)-alkenoate (1 mmol) was added and stirred overnight at 0°. For workup, Na_2SO_3 (1.5 g, 12 mmol) was added and the mixture warmed to 20° and extracted with AcOEt (3 ×). Washing with 2N NaOH (30 ml) and brine (50 ml)), drying (Na_2SO_4), and evaporation afforded the crude product, which was purified by LC (silica gel (50 g), petroleum ether/AcOEt 18:2 (200 ml), and petroleum ether/AcOEt 15:5 (400 ml)): > 0.9 mmol (> 90%) of methyl dihydroxyalkanoates **1**.

*Methyl (6*R*,7*R*)-6,7-Dihydroxy(6,7-$^2H_2$)-dodecanoate* ((6*R*,7*R*)-(6,7-$^2H_2$)-**1**) (from 'AD-Mix β'): ^{1}H-NMR ($CDCl_3$): 0.75 (*t*, *J* = 7, Me); 1.1–1.6 (*m*, 14 H, CH_2); 2.19 (*t*, *J* = 7, C*H*$_2$COOMe); 3.52 (*s*, COOMe); 0.05 H for signal of H–C(6) and H–C(7), *i.e.*, labeling 97%. ^{13}C-NMR ($CDCl_3$): 13.94 (C(12)); 22.52 (C(11)); 24.72; 25.08; 25.23; 31.78; 32.91; 33.28; 33.84; 51.46 (MeO); 73.29, 73.47, 73.63, 73.81, 73.95 (*m*, C(6), C(7)); 174.27 (C(1)).

The enantiomer (6*S*,7*S*)-(6,7-$^2H_2$)-**1** showed the same NMR spectrum.

3. *5-Hydroxydecano-4-lactones* (=*4,5-Dihydro-5-(1-hydroxyhexyl)furan-2(3*H*)-ones*) **2**. (4*R*,5*R*)- and (4*S*,5*S*)-**2** were prepared from methyl (4*E*)-dec-4-enoate by *Sharpless* AD and lactonization of methyl 4,5-dihydroxydecanoate in CH_2Cl_2/0.5M HCl 4:1 overnight at 20°. Enantiomers (4*R*,5*R*)- and (4*S*,5*S*)-**2** were oxidized at OH–C(5) with '*Dess-Martin* periodinane' [27] to yield enantiomers (4*R*)- and (4*S*)-5-oxodecano-4-lactone, resp., which were reduced with $NaBH_4$ in Et_2O to give (4*R*,5*R*)/(4*R*,5*S*)-**2** and (4*S*,5*S*)/(4*S*,5*R*)-**2**, resp. NMR-Signal assignments were performed by H,H-COSY and H,C-HETCOR.

(4*R*,5*R*)-**2** (*threo*): ^{1}H-NMR ($CDCl_3$): 2.46, 2.55 (2*m*, CH_2(2)); 2.07, 2.18 (2*m*, CH_2(3)); 4.36 (*ddd*, *J* = 7, 7, 5, H–C(4)); 3.51 (br. *m*, H–C(5)); 1.46 (*m*, CH_2(6)); 1.18–1.52 (*m*, CH_2(7), CH_2(8), CH_2(9)); 0.82 (*t*, *J* = 7, Me(10)); 2.30 (br. *d*, *J* = 7, OH–C(5)). ^{13}C-NMR ($CDCl_3$): 177.48 (C(1)); 28.62 (C(2)); 23.96 (C(3)); 82.98 (C(4)); 73.45 (C(5)); 32.82 (C(6)); 25.07 (C(7)); 31.58 (C(8)); 22.44 (C(9)); 13.92 (C(10)).

(4*S*,5*S*)-**2** (*threo*): ^{1}H- and ^{13}C-NMR identical to those of the enantiomer.

(±)-*erythro*-**2**: ^{1}H-NMR ($CDCl_3$): 2.47, 2.54 (2*m*, CH_2(2)); 2.08, 2.21 (2*m*, CH_2(3)); 4.38 (*ddd*, *J* = 7, 7, 4, H–C(4)); 3.86 (br. *m*, H–C(5)); 1.35 (*m*, CH_2(6)); 1.18–1.51 (*m*, CH_2(7), CH_2(8), CH_2(9)); 0.83 (*t*, *J* = 7, Me(10)); 2.95 (br. *d*, *J* = 7, OH–C(5)). ^{13}C-NMR ($CDCl_3$): 177.97 (C(1)); 28.64 (C(2)); 20.90 (C(3)); 83.04 (C(4)); 71.14 (C(5)); 31.80 (C(6)); 25.03 (C(7)); 31.58 (C(8)); 22.40 (C(9)); 13.87 (C(10)).

4. *Yeast Strain, Culture Conditions, Sampling, and Workup*. Yeast (*Saccharomyces cerevisiae* IfG 06136) was obtained from the culture collection of the 'Institut für Gärungstechnologie', Berlin. The organism was stored (4°) on worth agar-agar slants. For metabolic experiments, cells were cultivated in a medium (200 ml, pH 5.5) consisting of glucose (6.0 g/l), $MgSO_4$ (3.0 g/l), $(NH_4)_2SO_4$ (2.5 g/l), KH_2PO_4, L-alanine (2.5 g/l), and yeast extract (3.0 g/l) on a horizontal shaker at 17° and 100 rpm. The pre-culture (10 ml) was inoculated into 200 ml of fresh culture medium, and 30 mg (150 ppm) of each substrate, (*i.e.*, (6*R*,7*R*)-(6,7-$^2H_2$)-**1**, (6*S*,7*S*)-(6,7-$^2H_2$)-**1**, (±)-*threo*-(6,7-$^2H_2$)-**1a** and (±)-*erythro*-(6,7-$^2H_2$)-**1a**, in 250 µl of EtOH were immediately administered to different flasks. At certain times, *i.e.*, after 24, 48, 72, 96, 120, 144, 168, 216, and 268 h, aliquots (10 ml) of the culture broth were taken, internal standards (5 ppm of decan-1-ol, octano-5-lactone, and heptadecanoic acid) were added, the mixture was extracted with Et_2O (2 × 20 ml), the extract dried (Na_2SO_4), methylated with diazomethane, and converted to derivatives (silyl ether or trifluoroacetate) under standard conditions [28] if necessary.

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