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Hydroxylation of and Halogen Addition to the Carbon Carbon Double Bond of (R)-2-Hydroxy-3-enoic Acids

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Abstract: Various (R)-2-hydroxy-3-enoic acids and their derivatives have been subjected to epoxidations with peracids, dihydroxylations with osmium tetroxide or methylrhenium trioxide. Depending on the derivatives and reagents applied, the diastereomeric excesses (de) achieved were in the range of 8-80%. Based on the different addition mechanisms of osmium tetroxide and methylrhenium trioxide, all four possible 2,3-dihydroxy-γ-butyrolactones, i.e. (2R,3R,4R)-, (2R,3R,4S)-, (2R,3S,4R)- and (2R,3S,4S)-γ-butyrolactones with different substituents in the 4-position could be obtained.
The de values of the hydrogenation products of (R)-2-hydroxy-3-methyl-4-phenyl-3E-butenoic acid or its derivatives with a nonchiral Wilkinson catalyst depended noticeably on the derivatives used.
High and extremely high stereoselectivities were observed with the halolactonization of (R)-2-hydroxy-3-enoic acids with N-bromosuccinimide or iodine leading to (2S,3R,4S)-2-hydroxy-3-halogeno-γ-butyrolactones. Br_2-addition formed (2S)-2-hydroxy-3,4-dibromo carboxylic acids with stereoselectivity markedly depending on the derivatives of (R)-2-hydroxy-4-phenyl-butenoic acid applied and on the substituents at the double bond.

Introduction

Many different types of (R)-2-hydroxy carboxylic acids can be obtained by the bioreduction of the corresponding 2-oxo carboxylic acids with resting cells of *Proteus vulgaris* at the expense of hydrogen gas and/or formate.[1] Recently, we showed an efficient and highly stereoselective preparation of various (R)-2-hydroxy-3-enoic acids and (R)-2-hydroxy-4-oxo carboxylic acids from the corresponding 2-oxo carboxylic acids by this biocatalyst.[2] The former products are on one hand allylic alcohols, and on the other hand unsaturated carboxylic acids. Reactions of chiral and achiral allylic alcohols or unsaturated carboxylic acids have been studied in great detail.[3-10] Stereoselective epoxidations,[3] like the Sharpless epoxidation[4] and dihydroxylation,[5] have been achieved with great success. In some cases, halogen additions to unsaturated carboxylic acids[6] or to 3,4-unsaturated alcohols[7] lead to halogenolactones or halogenated cyclic ethers. But the addition to allylic alcohols gives α-halogeno-epoxides in poor yields.[8]

The hydrogenations of open chain unsaturated compounds with a chiral center usually does not result in good diastereoselectivities unless chiral catalysts are applied.[9] With ring systems better results have been obtained.[10] However, it seems that enantiomerically pure (R)-2-hydroxy-3-enoic acids have not been applied for such addition reactions.[11] Therefore, we studied the diastereoselectivity of various reactions on the carbon carbon double bond of different (R)-2-hydroxy-3-enoic acids or derivatives thereof (Scheme 1) and synthesized a series of chiral building blocks with two or in most cases three chiral centers.

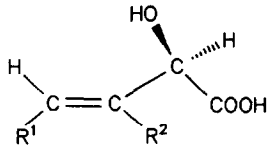
Three types of reactions were chosen: (i) Through epoxidations and hydroxylations, the (R)-2-hydroxy-3-enoic acids were converted into epoxides and 2,3,4-trihydroxy carboxylic acids which were isolated as γ-butyrolactones through acid workup (Scheme 2). (ii) By addition of bromine or iodine using various halogenation agents, open chain (S)-2-hydroxy-3,4-dibromo carboxylic acids as well as γ-butyrolactones containing one halogen atom in the C-3 position were obtained with excellent diastereoselectivity (Scheme 3). (iii) Various derivatives of (R)-2-hydroxy-3-methyl-4-phenyl-3-enoic acid were hydrogenated with an achiral Wilkinson catalyst in order to study the diastereoselectivity of the hydrogenation (Scheme 4).

Results and Discussion

Protecting groups of the (R)-2-hydroxy-3-enoic acids. According to Scheme 1, only the E-isomers of the (R)-2-hydroxy-3-enoic acids were applied. For some of the conducted reactions, the carboxylic and/or the hydroxyl group were derivatized or protected. The methods by which the carboxylic group of the (R)-2-hydroxy-3-enoic acids were esterified depended on the substituent in the 4-position of the acids. With a phenyl residue, i. e. **1** and **5**, satisfying yields of the methylester were obtained with dry methanol in the presence of HCl. However, under the same conditions a methyl residue in the 4-position as in **4** led to by-products which were probably diastereomeric lactones.[12] This side reaction can be avoided by using methanol and a cation exchange resin in its protonated form as catalyst for the esterification.

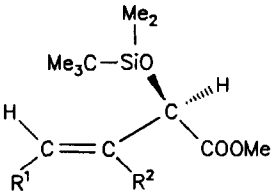
In order to protect the hydroxyl group of the (R)-2-hydroxy-3-enoic acid esters, many methods are possible. The tosylation of **1** methylester with tosyl chloride in pyridine/tetrahydrofuran led, according to HPLC analysis, to three products, which were not isolated and characterized. Also, treatment with 2-methoxyethoxymethyl chloride in pyridine/tetrahydrofuran did not result in a pure product. With trimethylsilyl chloride and pyridine in dry ether or tetrahydrofuran, the expected silyl ether of **1** methylester was formed, but it was rather labile, hydrolyzing even during HPLC analysis with CH_3OH/H_2O (pH 2.0) as eluent. The isolated trimethylsilyl ether partly hydrolyzed also under acidic conditions during an epoxidation with peracids. Reacting **1** methylester with t-butyldimethylsilyl chloride and imidazole in dry acetonitrile led to the corresponding silyl ether which was isolated in almost quantitative yield. It was stable in a pH 2.0 aqueous solution at room temperature for several hours.

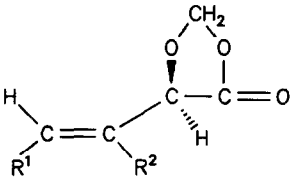
The simultaneous protection of the carboxyl and hydroxyl group was achieved by methods from Seebach et al.[13] using aldehydes. In order to prevent



Besides the free acids the methyl and isopropylesters, 2-(t-butyl-di-methylsiloxy)- and 1,3-dioxolanone-4 derivatives were applied.

	R^1	R^2
1	Ph	H
2	p-ClPh	H
3	2-thienyl	H
4	Me	H
5	Ph	Me
6	PhCH=CH	H





Scheme 1. (R)-2-Hydroxy-3-enoic acids and derivatives used in various reactions.

the formation of an additional chiral center leading to diastereomers, formaldehyde was applied for the formation of 5-substituted 1,3-dioxolanones-4 (Scheme 1). The reaction of acids **1** and **5** with formaldehyde catalyzed by a trace of p-toluenesulfonic acid resulted in good yields.

Epoxidation. The results of epoxidation of **1**, its methylester and the methylester of **5** with m-chloroperbenzoic acid (MCPBA) or mono-perphthalic acid (MPPA) (Scheme 2) are shown in Table 1. The products were not isolated in separated form. The de values were determined by HPLC analyses. The stereochemical assignment was concluded from those determined for the γ-butyrolactones to which the epoxides were transformed by acidic workup. Since the ratio of the diastereomeric lactones formed from the epoxides is the same as that of the original epoxides, we assume that the formation of the lactones is a result of an acid catalyzed intramolecular reaction of the protonated epoxide and the alkoxycarbonyl or carboxyl group (Scheme 2).

Following this assumption, it is concluded that the isolated diastereomeric mixture of the epoxides of **1** methylester consisted of 73% (2R,3R,4R)- and 27% (2R,3S,4S)-2-hydroxy-3,4-epoxy-4-phenylbutanoic acid methylester. This mixture was converted into lactones leading to 73% **7** and 27% **7a** as shown by HPLC analysis. This implies a stereospecific reaction. The configuration of lactone **7** was assigned by NOE (Nuclear-Overhauser Effect) analyses. The marked NOE of **7** between H_{C2}/H_{C4} and the negligible effect between H_{C2}/H_{C3} and H_{C3}/H_{C4} indicate that H_{C2} and H_{C4} are located at the same side of the lactone ring and H_{C3} at the other. Their large coupling constants J_{HC2HC3} and J_{HC3HC4} of 10 Hz are in agreement with this assumption. Since C-2 has R-configuration from the biocatalytic synthesis,[2b] the structure of the lactone **7** is assigned as (2R,3R,4S)-2,3-dihydroxy-γ-butyrolactone originating from the (2R,3R,4R)-2-hydroxy-3,4-epoxide of **1** methyl ester if an inversion at C-4 is assumed in the lactonization. The corresponding arguments can also be used for lactone **7a**. The observed relatively small coupling constants, J_{HC2HC3} and J_{HC3HC4} of 2.8 Hz and 3.7

Table 1. Products of the epoxidation of (R)-2-hydroxy-3-enoic acids **1** and **5** or methylesters.

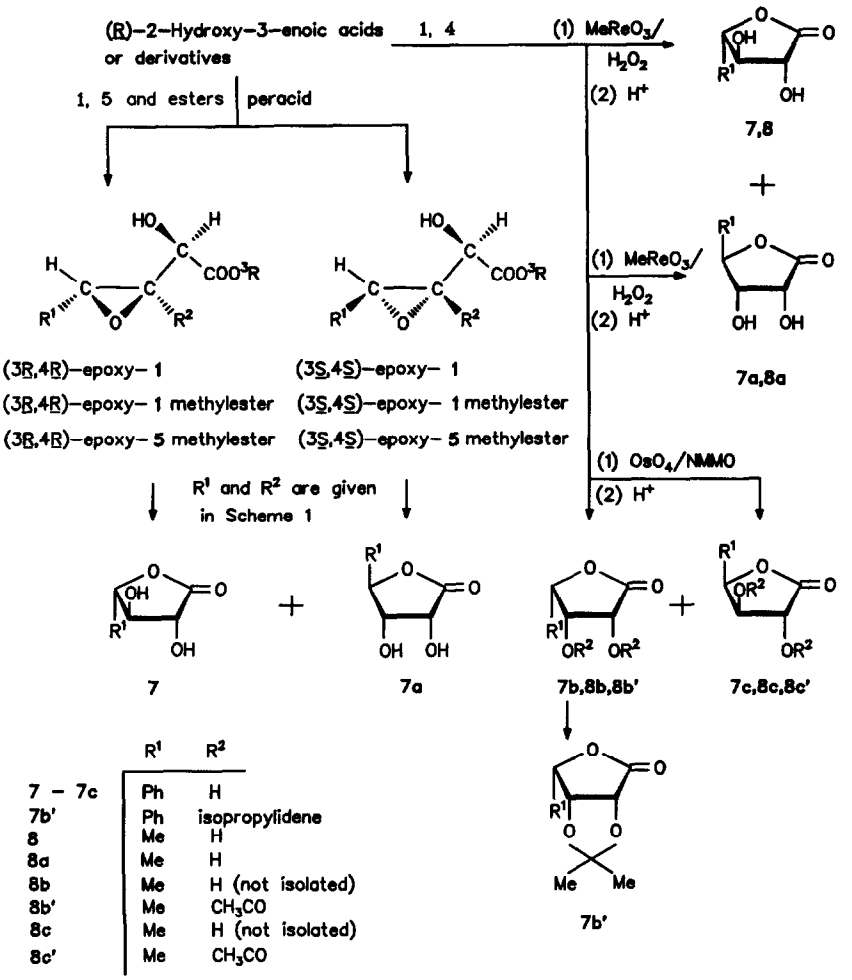
Starting material	Major and minor products	Reagent	Yield(%)[a]	de(%)[b]
1	(3R,4R)-epoxy **1** (3S,4S)-epoxy **1**	MCPBA	-[c]	8
1 methylester	(3R,4R)-epoxy **1** ester (3S,4S)-epoxy **1** ester	MCPBA	89	46
1 methylester	(3R,4R)-epoxy **1** ester (3S,4S)-epoxy **1** ester	MPPA	80	30
5 ester	(3,4)-epoxy **5**[d]	MCPBA	75	7
5 ester	(3,4)-epoxy **5**[d]	MPPA	67	23

[a] As diastereomeric mixture; [b] Determined by HPLC; [c] Product not isolated; [d] Stereochemistry not determined.

Hz, respectively, are in accordance with the configuration (2R,3S,4R)-2,3-dihydroxy-γ-butyrolactone as depicted in Scheme 2. Lactone **7a** was formed from the 2-hydroxy-3,4-epoxide of **1** methylester, for which the (2R,3S,4S) configuration is assumed.

Dihydroxylations. The dihydroxylations of various (R)-2-hydroxy-3-enoic acids and their derivatives with OsO_4/NMMO (N-methylmorpholine-N-oxide) or $MeReO_3/H_2O_2$, followed by acid treatment, produced diastereomeric mixtures as shown in Scheme 2. Based on HPLC analysis, a complicated mixture of products was formed with $MeReO_3$ as catalyst. Presumably, it consisted of diastereomeric epoxides, trihydroxy compounds and lactones. After treatment with 2N sulfuric acid, only two diastereomeric lactones were present as shown by HPLC. According to the literature [14], with $MeReO_3$ as catalyst, cyclohexene is converted at first to an epoxide which opens to a diol through catalysis also caused by $MeReO_3$. Reacting **1** methylester with $MeReO_3$ led to lactone **7** and **7a** from which **7** was obtained with 41% overall yield after two recrystallizations from hexane/$CHCl_3$. This lactone is identical with that obtained from the major epoxide diastereomer of the epoxidation of **1** methylester with MCPBA (Scheme 2).

The hydroxylation of **1** or its methylester with OsO_4 probably yielded a mixture of trihydroxy carboxylic acids and lactones, which was converted completely into lactones by acidic workup. However, a higher reaction temperature (80°C) was needed for the formation of **7b**. As will be shown later this is probably because all three groups bulkier than hydrogen are on the same side of the lactone ring causing some difficulties for the formation of the lactone. This lactone was isolated with 40% overall yield after recrystallizations from hexane/chloroform. The lactones **8b** and **8c** were difficult to isolate from the reaction mixture because of their good water



Scheme 2. Epoxidation and dihydroxylation of various (2R)-hydroxy-3-enoic acids or derivates.

solubility. Therefore, the reaction mixture was vacuum dried and treated with acetic anhydride/pyridine to convert the lactones to their diacetate derivatives. These were isolated through preparative HPLC applying a Nucleosil RP-18 column.

The configurations of lactones **7** and **7b**, and the diacetylated lactones **8b'** and **8c'** were identified by NMR analyses. As already mentioned, the products obtained from **1** with $MeReO_3$ are identical with those formed by the epoxidations of **1** or its methylester with MCPBA. That means, they are **7** and **7a**. Lactone **7b** was identified as an all cis-lactone on the basis of its positive NOEs between any two of the three protons H_{C2}, H_{C3} and H_{C4} as well as on the basis of its coupling constants, J_{HC2HC3} and J_{HC3HC4}, of 2.9 Hz. Lactone **7b** has the same ^{13}C-NMR spectrum as the one synthesized from D-ribonolactone by Beer et al..[15] The formation of the isopropylide-

ne derivative **7b'** also indicates the 2,3-cis-dihydroxy structure.
Applying OsO_4 and $MeReO_3$ hydroxylation and peracid epoxidation, all four possible stereochemically different lactones **7/7a**, and **7b/7c** could be obtained (Scheme 2). This is due to different reaction mechanisms, i. e., OsO_4 catalyzes cis- and $MeReO_3$ trans-hydroxylation. Peracids lead to cis epoxidation. Two of the four lactones were isolated as main products, which can be used in further synthesis as described by Beer et al..[15]
The extent of the diastereoselectivities of the epoxidations and hydroxylations depends upon the reagents used and the substituents of the double bond (Table 1 and 2). For the epoxidations of **1** methylester, the selectivity is better with MCPBA than with MPPA, and for the methylester of **5** the opposite is true. The selectivity of the epoxidations of **1** methylester with MCPBA or MPPA is clearly better than that of the branched methylester of **5**. The epoxidation of acid **1** is hardly stereoselective in contrast to its methylester.
The hydroxylations of acid **1**, its methylester, its dioxolanone and 2-t-butyldimethylsilyl ether of **1** methylester, as well as the methylesters of **4** and **5** with OsO_4 or $MeReO_3$ can be summarized as follows: i) Both catalysts show similar selectivity but form products of different configurations; ii) The free acid **1** is generally less selectively hydroxylated than its methylester; iii) The protection of the C-2 hydroxyl group as t-butyldimethylsilyl ether leads to less selective hydroxylations. The same is true for the 1,3-dioxolanone of **1a**. The hydroxylations of **1** methylester with both catalysts show a similar selectivity as that observed for epoxidation with MCPBA. An advantage of the $MeReO_3$ hydroxylation as compared to the peracid epoxidation is the easier isolation of the lactones from the reaction mixture.
Common procedures for Sharpless epoxidation of **1** and **5** methylesters failed. The expected products and their behavior in HPLC were known from the epoxidation of **1** and **5** methylester with peracids, but they could not be observed after conducting the Sharpless[4a] or modified Sharpless[4c,4d] epoxidation with **1** and **5** methylesters in yields more than 5 - 10%. We assume that the ester group prevents the formation of a proper complex for the Sharpless epoxidation.[4b]

Halolactonizations. According to Scheme 3, the halolactonizations of various (R)-2-hydroxy-3-enoic acids were conducted by using N-bromo-succinimide (NBS) or I_2/KI.
Acids **1,2,3,5** and **6** were converted to 2-hydroxy-3-bromo-γ-butyrolactones **9-13** with excellent stereoselectivities by using a slight excess of NBS in dimethylformamide (DMF). According to HPLC the reaction mixture usually contained a major product (90 - 95%) of the two diastereomeric lactones.
It could easily be purified by recrystallizations of the reaction mixture from hexane/chloroform. The configurations of the lactones **9-13** were established as follows: The IR absorptions of the carbonyl groups of all bromolactones were in the region 1780-1802 cm^{-1}, indicating a five ring lactone. A four ring lactone should have an IR absorption near or above 1830 cm^{-1}, according to Van Tamelen and Shamma[6a] and Barnett and Sohn.[6b] The NOE between H_{C2}/H_{C4} of **9** and **11** is positive (6-14% signal increase), whereas those between H_{C2}/H_{C3} as well as H_{C3}/H_{C4} of the same compounds are

Table 2. Dihydroxylations of (R)-2-hydroxy-3-enoic acids, methylesters or other derivatives with osmium tetroxide or methylrhenium trioxide.

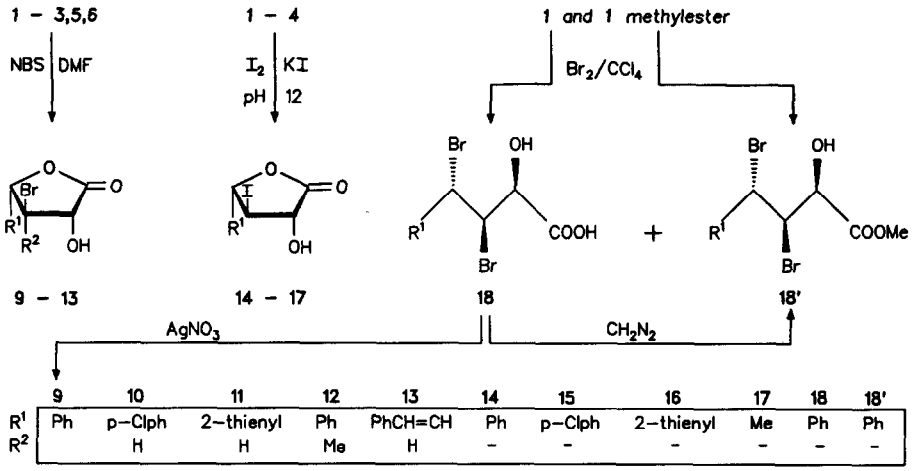
Starting material	Product	Catalyst	Yield(%)[a]	de(%)[a]
1	**7b/7c**	OsO_4	-	20
1	**7/7a**	$MeRhO_3$	-	20
1 ester	**7b/7c**	OsO_4	41(81[b])	50
1 ester	**7/7a**	$MeReO_3$	42(86[b])	40
O-t-butyldimethyl-silyl-**1** ester	**7b/7c**	OsO_4	-	34
1,3-dioxolanone-4 of **1** ester	**7b/7c**	OsO_4	-	28
1,3-dioxolanone-4 of **1** ester	**7/7a**	$MeReO_3$	-	25
4 ester	**8b/8c**	OsO_4	34[d]	20
4 ester	**8/8a**[c]	$MeReO_3$	-	28
5 ester	-	OsO_4	-	80
5 ester	-	$MeReO_3$	-	58

[a] If no yield is given the products were not isolated. They were converted into lactones and their ratio determined by HPLC. In some cases derivatives such as **7b'** or **8b'** were isolated. [b] Yield of the diastereomeric mixture. [c] Configuration assumed on the basis of the assignments of **7** and **7a**. [d] Yield of the diacetylated lactones.

almost zero, indicating that the protons H_{C2} and H_{C4} are at the same side of the lactone ring and H_{C3} at the other. The coupling constants of 10 Hz between H_{C2}/H_{C3} and H_{C3}/H_{C4} support the conclusions from the NOE analyses. Since the known configuration of the C-2 is not changed during bromolactonization, the configurations of the other centers are also fixed due to the above mentioned correlations.

For the iodolactonization, the acids **1-4** were dissolved in a potassium hydroxide solution at pH 12 and converted into iodolactones **14-17** by adding I_2/KI. Only one diastereomer was detected. The configurations of the iodolactones were established by the same methods applied for the assignment of the bromolactones using the IR spectroscopy, NOEs and coupling constants determined by ^{1}H-NMR spectroscopy. Therefore, all lactones (**9-17**) depicted in Scheme 3 have, according to the Cahn-Ingold-Prelog nomenclature 2S,3R,4S-configuration.[16]

Br_2-addition. The bromine addition to acid **1**, its methylester and 1,3-dioxolanone, as well as to **4** methylester was carried out in tetrachloromethane at -20°C (Scheme 3). According to HPLC analyses, **4** methylester gave a main product corresponding to only 50% de and 1,3-dioxolanone of **1** resulted in products with 60% de. Remaining results are listed in Table 3. The dibromo compounds were isolated in form of diastereomeric mixtures. They are usually solid compounds at room temperature. The Br_2-addition to **1** in CCl_4/diethyl ether (1:1) led to three products. In addition to two minor products, only one dibromo acid **18** was detected. It was determined by HPLC-analyses that one of the minor products seemed to be lactone **9**, having identical retention time on HPLC with that formed by treatment of



Scheme 3. Halogenation of various (R)-2-hydroxy-3-enoic acids or derivatives. Only products which are formed in high de values are shown. **18** can be converted to **9** by $AgNO_3$ and to **18'** by CH_2N_2. This leads to the assignment of the configuration.

1 with NBS. After recrystallizations of the ternary mixture from chloroform, only the dibromo acid **18** was isolated as needles in 45% yield. As determined by NMR spectroscopy, the bromine addition to **1** methylester resulted in a diastereomeric mixture of 90:10 when conducted at 0°C and 95:5 at -20°C, respectively. The main diastereomer was purified after two recrystallizations from ethanol/water (1:1). The configuration of the main product was determined by the reactions shown for **18** in Scheme 3. Acid **18** was methylated to its methylester, which had the same ^1H- and ^{13}C-NMR spectra as the methylester **18'** obtained by Br_2-addition to **1** methylester, proving the configurations of the two dibromo compounds at all three chiral centers are either the same or mirror images. But since the chiral center at C-2 has the same configuration as known,[1] the two methylesters must be identical. On the other hand, acid **18** was converted to lactone **9** by treatment with $AgNO_3$/pyridine in tetrahydrofuran. Since this reaction also forms by-products (surely not the diastereomeric bromolactone of **9a** according to HPLC analyses), lactone **9** was not isolated, but identified by HPLC to be the same lactone which resulted from the NBS-bromolactonization of **1**.

The formation of lactone **9** can be explained by assuming an attack of the carboxylate group on C-4, with release of Br^- trapped by Ag^+ as AgBr. Even though the reaction can proceed either via an S_{N1} or an S_{N2} mechanism, the chiral center at C-3 should not be changed in either case. Therefore, C-3 of the acid **18** must have, like that of the lactone **9**, an S-configuration. As well-known, Br_2-addition to a carbon carbon double bond in nonpolar solvents always occurs in an anti-fashion.[17] Therefore, the configuration at C-4 of acid **18** should be S, as well as that of the methylester **18'**. From these assumptions it can be concluded that the conversion **18** --> **9** proceeds via retention of configuration.

Table 3. Halolactonizations of various (R)-2-hydroxy-3E-enoic acids according to Scheme III.

Starting material	Product	Yield(%)[a]	de(%)[b]	mp.(°C)	IR(cm^{-1})[c]	α_D[d]
Bromolactonization						
1	9	55	80	120-2	1790	-21.9°(0.034)
2	10	60	88	121	1802	-45.6°(0.05)
3	11	83	90	106-10	1800	-68.9°(0.035)
5	12	71	90		1780	-18.3°(0.12)
6	13	85	80	125-9	1765	-37.0°(0.07)
Br_2 addition						
1	18	45	>96			+60.1 (0.070)
1[e]	18'	98	90			+50.7 (0.069)
1[f]	-	82	60			+33.9 (0.068)[g]
4[e]	-	87	50			-18.0 (0.069)[g]
Iodolactonization						
1	14	71	>96	137-8	1788	-24.2°(0.043)
2	15	58	>96	101-3	1775	-39.8°(0.034)
3	16	64	>96	121-3		-82.2°(0.060)
4	17	53	>96	143-7	1780	-26.6°(0.048)

[a] The yields are obtained after recrystallization of the products; [b] The de values were obtained by HPLC-analyses of the reaction solution after disapearance of the starting material; the de value of 18a is obtained from the reaction at 0°C, that of at 20°C is 80%. The de values of the iodolactonization reactions indicate that no other diastereomer is detected; [c] IR-absorption of the lactone carbonyl groups; [d] The $[\alpha]_D$ values were determined at 20°C in methanol. Concentration of the lactones are given as mol/l; [e] Methylester; [f] 1,3-dioxolanone-4 derivative of 1; [g] Measured for the diasteromeric mixture.

The diastereoselectivity of Br_2-addition depends on the substituents of the double bond. For 1 and the methylesters of 1 and 4 the larger phenyl group makes the reaction more selective than the methyl group at C-4. Whereas, the Br_2-addition to 1 gives only one of the two possible dibromo diastereomers, the Br_2-addition to 1 methylester and the 1,3-dioxolanone of 1 show de values of 90% and 60%, respectively. The reaction temperature also plays a role. At lower temperature, the Br_2-addition is more selective.

As can be seen (Table 3), the iodolactonization of acids 1-4 gives better diastereoselectivity than that of the bromolactonization. After iodolactonization no other diastereomer could be detected. It is known that in such an addition reaction to a carbon carbon double bond a three ring halogen cation intermediate is formed.[18] This is attacked by another nucleophile present in the reaction solution. In our case, the attacking agent is the carboxylate group of the (R)-2-hydroxy-3-enoic acids. Such "neighboring group" involved halogen addition reactions have also been described by several authors in studying the halolactonization of unsaturated carboxylic acids.[6-8] It may be, that in the case of the bulkier iodine the intermediate three ring halogenium ion for halogen addition to a carbon car-

bon double bond[6h,18] only allows the formation of one of the possible lactones during iodolactonization.

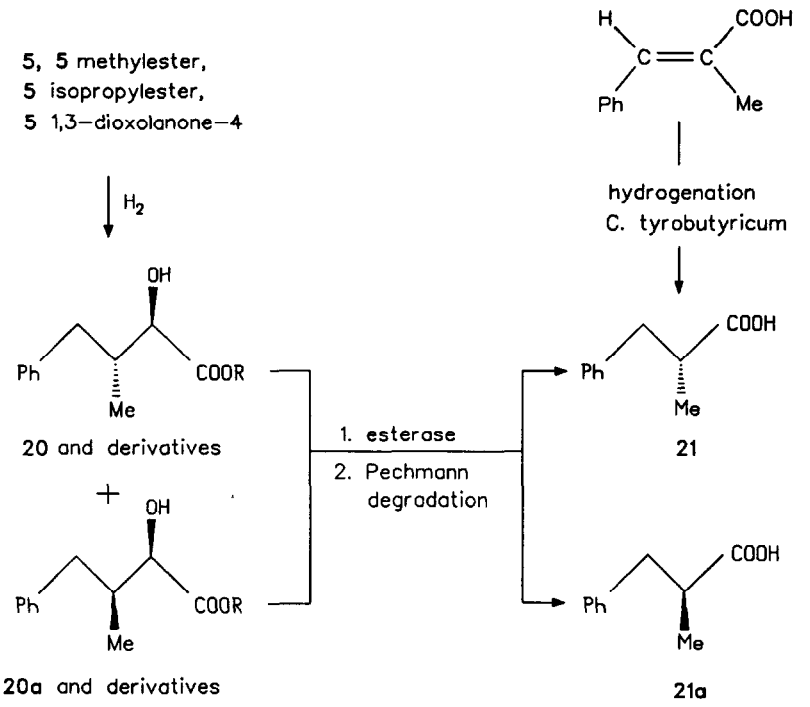
Hydrogenation. The hydrogenations of **5**, its methyl and isopropyl ester and the 1,3-dioxolanone of **5** were carried out with hydrogen gas and $Rh(Ph_3P)_3Cl$ as catalyst with a molar catalyst/reagent ratio of 1:5 to 1:10.

The diastereomeric products of the hydrogenation of **5** and its derivatives could be separated by HPLC. The de values of the hydrogenated esters were determined by HPLC analyses after being hydrolyzed to the acids **20** and **20a**. The de values of the hydrogenated methyl and isopropyl ester were also determined by ^{1}H-NMR analyses, since the corresponding signals from the diastereomeric products have different chemical shifts. The de values were calculated from the resulting integrals. The hydrogenated isopropyl and methylesters were isolated as pure products. The de ratio of the hydrogenations using other derivatives of **5** were determined by NMR and HPLC in the reaction solutions without isolating the products. The configuration of the hydrogenation products of **5** isopropylester was determined as shown in Scheme 4.

In order to prevent racemization or other side reactions, the isopropyl esters **20/20a** (85 : 15) were hydrolyzed by using an esterase from bovine liver at pH 8.0. The isolated acids **20/20a** were degraded to the one C-atom shorter acids **21/21a** with a ratio of 85 : 15 as shown later. According to the literature this Pechmann reaction is effective with short chain 2-hydroxy carboxylic acids.[19] However, the degradation of a long chain 2-hydroxy carboxylic acid had to be optimized.

Under the usual conditions[19] the main product of the degradation of **20/20a** was benzoic acid. With a molar ratio of $Ce(SO_4)_2$/ hydroxy acid 5:1, a sulfuric acid concentration of 0.3N at 80-100°C, the expected decomposition product was obtained with 70% yield.

The acids **21/21a** were separated on a cellulose triacetate column. In order to determine the configuration of the enantiomers **21/21a** obtained from **20/20a**, the racemic mixture of **21/21a** was prepared by Pd/C catalyzed hydrogenation of 2-methyl-3-phenyl-2E-propenoic acid, leading to a 1:1 mixture of **21** and **21a**. (R)-2-Methyl-3-phenylpropionate was synthesized by a biocatalytic hydrogenation of 2-methyl-3-phenyl-2E-propenoic acid with *C. tyrobutyricum* .[20] For this biocatalyst, it has been shown in many cases that only the R-enatiomer is formed within the limits of the analytical procedure.[20a-c] Under the selected conditions of HPLC on a cellulose triacetate column the retention times of the Pechmann degradation products **21/21a**, which appeared in the ratio of 85 : 15, were 87 min. and 75 min., respectively. The racemic form of **21/21a** gave the same retention times but with the ratio of 50 : 50. Finally, the pure R-form obtained independently by biocatalytic hydrogenation had a retention time of 87 min.. Therefore, it is conclusive that the Pechmann degradation products are a mixture of 85% (R)- and 15% (S)-2-methyl-3-phenyl-propionic acid. Since the chiral center at C-3 of the **20/20a** isopropyl ester was not changed during the hydrolysis and degradation reaction, the acids **20** and **20a**, their methyl as well as isopropyl esters must have the configuration (2R,3R) and (2R,3S), respectively. The diastereomers **20** and **20a** were formed from **5**, **5** methyle-



Scheme 4. Hydrogenation of **5** and derivatives. Determination of the diastereomers.

ster, **5** propylester and **5** 1,3-dioxolanone-4 in the following ratios: 53:47, 82:18, 85:15 and 70:30.

Concluding Remarks

Many publications are dealing with addition reactions to chiral and achiral allylic alcohols as well as to unsaturated acids. The here studied chiral 2-hydroxy-3-enoic acids are a special type of an allylic alcohol and an unsaturated acid which have hardly been examined yet, because they were not readily available. Compared with other allylic alcohols, they behave differently with respect to epoxidation catalysts as used by Sharpless et al..[4] The high degree of functionality and chirality of the compounds render them useful as chiral synthons. A known reaction is the synthesis of antibiotics using γ-butyrolactones[15] including **7b**. Especially the 3-halogeno-γ-butyrolactones possessing three chiral centers and four functional groups can be obtained from the corresponding 2-oxo-3-enoic acids in two steps in high yields and excellent stereoselectivity. As already shown, *P. vulgaris* can also be used for the preparation of (S)-2-hydroxy carboxylic acids. By selective dehydrogenation of a racemic 2-hydroxy carboxylic acid, (S)-2-hydroxy carboxylic acid and the corresponding 2-oxo carboxylic acid are formed.[2a] As will be described elsewhere, we synthesized by this route the enantiomer of **14**. Another possibility to use the compounds described here may be the conversion of suitably selected

substituents in the 4-position after a compatible addition reaction to the carbon carbon double bond.

EXPERIMENTAL

Materials and instruments. The (R)-2-hydroxy-3-enoic acids 1-6 were obtained always in the E-form as products of the biocatalytic reduction of their corresponding 2-oxo-3-enoic acids with resting cells of *Proteus vulgaris*.[2] The chemicals and solvents of analytical purity purchased from Aldrich, Merck and Sigma were used without further purification. The $MeReO_3$ was a gift. ^1H- and ^{13}C-NMR spectra and NOE (Nuclear-Overhauser Effect) data were measured in deuterated solvents on Bruker WP 200, Bruker AM 360 and JEOL FX 90 spectrometers. Chemical shifts are given in ppm relative to an internal Me_4Si standard. Melting points were obtained on a Fisher-Johns apparatus and are not corrected. MS spectroscopy was carried out on a Varian MAT CH5 instrument. Elemental analyses were performed by a C,H,N-Analysis automat of Hepaeus (D-6450 Hanau), and optical rotation was measured on an ORD-Spectral photometer J5 from Jasco (J-Tokyo, Japan).

HPLC analysis. If not mentioned otherwise HPLC columns (4x250 mm) filled with 10 µm Nucleosil RP 18 (Macherey and Nagel, D-5160 Düren) were applied. Besides this, a cellulose triacetate column (CTA, 7.5x250mm, Merck, D-6100 Darmstadt), was used. The solvents used for HPLC were double distilled. For preparative purposes a Nucleosil RP 18 column 20x250 mm was used. Depending on the hydrophobicity of the compounds 0.1% aqueous phosphoric acid containing 10-60% methanol was used as an eluent with a flow rate of 1-2 ml/min.. The refractive index and uv absorption were simultaneously recorded. Compounds without groups absorbing at wave lengths above that of the carboxylate group the absorption at 214 nm was detected. For analyzing unknown concentrations calibration curves were established. Usually the compounds were purified until they were homogeneous by HPLC. For comparing compounds not only the retention times were determind but also the mixtures were coinjected.

Determination of diastereomeric excesses (de values). The diastereomeric products could be mostly separated by HPLC columns (Nucleosil RP-18 or cellulose triacetate). The de values were obtained by measuring the chromatography peak area ratios from the recorder. Some of the de values were determined by ^1H-NMR analysis, if the separation by an HPLC column was not satisfying. If both methods were available, the results from both were usually used for comparison.

Esterification of (R)-2-hydroxy-3-enoic acids. In 20 ml dry alcohol, which was bubbled with HCl gas for several min., 10 mmol (R)-2-hydroxy-3-enoic acid was dissolved and stirred for 30-60 min., until the acid disappeared according to HPLC analysis on a Nucleosil RP-18 column. The alcohol was then evaporated in vacuum at 20°C. The residue was dissolved in 50 ml diethyl ether, washed with H_2O, 1N $NaHCO_3$ solution and again H_2O and dried over $MgSO_4$. After filtration and evaporation of the diethyl ether, an oily residue was usually obtained, which crystallized slowly at 0°C .

Methylester of 1 was obtained in 90% yield after crystallization from (hexane/-chloroform). Mp. 39-41°C; $[\alpha]_D$ = -67.7° (0.050 M, $CHCl_3$) or -59.4° (0.050 M, MeOH); ^1H-NMR ($CDCl_3$, AM-360): δ 7.3 (m, 5H, arom.), 6.8 (dd, J=15.8Hz, 1.54Hz, -HC=CH-), 6.25 (dd, J=15.8Hz, 5.7Hz, -HC=CH-), 4.85 (dd, J=5.7Hz, 1.54Hz, -C(OH)H-), 3.82 (s, $COOCH_3$), 3.3 (br, OH). ^{13}C-NMR ($CDCl_3$, AM-360): δ 173.8, 136.1, 132.4, 128.6, 128.1, 126.7, 125.3, 71.3, 53.0.

5 Methylester. Yield after crystallization 88% (hexane/chloroform); mp. 45-46°C; ^1H-NMR($CDCl_3$, WP-200): δ 7.24 (m, 5H, arom.), 6.40 (s, -CH=CMe-), 4.40 (s, -C(OH)H-), 3.95 (s, 3H, $COOCH_3$), 1.68 (s, 3H, -CH=C(CH_3)-).

5 Isopropyl ester: Yield 78% as an oil, pure as judged by HPLC; ^1H-NMR($CDCl_3$, WP-200): δ 7.35 (m, 5H, arom.), 6.75 (s, 1H, -CH=C(Me)-), 5.10 (hept., 1H, -$CHMe_2$), 4.80 (s, 1H, C(OH)H-), 3.3 (br, OH), 1.95 (s, 3H, -CH=C(CH_3)-), 1.41 (d, 3H, -C(CH_3)CH_3), 1.39 (d, 3H, -C(CH_3)CH_3). ^{13}C-NMR($CDCl_3$, FX 90): δ 173.1, 136.9, 134.6, 129.4, 128.8, 128.0, 126.7, 76.6, 69.9, 21.6, 21.5, 13.4.

4 Methylester. Amberlite GC-400 (ion-exchanger resin, Aldrich) (1.5g), pre-washed with 3x10 ml conc. hydrochloric acid and then with 2x10 ml dry methanol, was mixed with 5.0 g 4 in 20 ml dry methanol. After 24 h stirring at room temperature, the starting material has disappeared. Workup as described above for 1 methylester led to 4.5 g of an oily residue. It was contaminated with about altogether 10% of various by-products probably a dimer and lactones. Further purification did not result in better purity.

(R)-2-(t-Butyldimethylsiloxy)-4-phenyl-3-butenoic acid methylester. A mixture of 2.0 mmol 1 methylester, 3.0 mmol imidazole and 2.5 mmol t-butyldimethylsilyl chloride was dissolved under a stream of dry nitrogen gas in 0.3 ml acetonitrile and stirred for 1 h at room temperature. The white imidazole hydrochloride precipitate was filtered off and washed with 3x5 ml diethyl ether. The filtrate, combined with the ether solution, was washed with 10 ml H_2O, aqueous acetic acid (pH 3.5), 0.1 N $NaHCO_3$, again with H_2O, and dried over Na_2SO_4. After filtration and evaporation of the solvent, 614 mg colorless, oily product was obtained corresponding to quantitative yield. $[\alpha]_D$ = -27.2°(0.060 M, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, AM-360): δ 7.39 (d, J=7.8Hz, 2H, arom.), 7.32 (dd, J=7.8Hz, 2H, arom.), 7.25 (m, 1H, arom.), 6.75 (d, J=15.8Hz, 1H, -CH=CH-), 6.30 (dd, J=15.8Hz, 5.6Hz, 1H, -CH=CH-), 4.9 (d, J=5.6Hz, 1H, -C(OH)H-), 3.75 (s, 3H, $-COOCH_3$), 0.95 (s,9H, $-C(CH_3)_3$), 0.14 (s, 3H, $-Si-CH_3$), 0.12 (s, 3H, $-Si-CH_3$). ^{13}C-NMR($CDCl_3$, AM-360): δ 172.3, 136.3, 131.5, 128.6(2xC), 127.9, 126.7(2xC), 126.5, 73.2, 52.2, 25.7(3xC), 18.5, -5.0, -5.1.

Reaction of (R)-2-hydroxy-3-enoic acids with para-formaldehyde. In analogy to the literature [13] a mixture of 10 mmol 2-hydroxy-3-enoic acid, 50 mmol paraformaldehyde and 50 mg p-toulene sulfonic acid was dissolved in 48 ml dry benzene (distilled from sodium) and heated under reflux with a water-separator. After 5h, the starting material disappeared as seen by HPLC analysis. The solution was diluted with 30 ml diethyl ether and washed with 20 ml H_2O, 0.1N $NaHCO_3$ solution, again with water and dried over Na_2SO_4. The solid residue was recrystallized from hexane/$CHCl_3$ (4:1) and 8-9 mmol white crystals were obtained.

(R)-5-(2'E-phenylethenyl)-1,3-dioxolanone-4 derivative of 1. Yield after recrystallization from $CHCl_3$ 91%; $[\alpha]_D$ = + 21°(0.30M, CH_2Cl_2); mp. 68-70°C; MS: M^+ 194; IR(film): 1750 cm^{-1} (C=O of the lactone ring); ^{1}H-NMR(DMSO, AM-360): δ 7.32 (m, 5H, arom.), 6.83 (dd, J=16.0Hz, 1.5Hz, 1H, -CH=CH-), 6.18 (dd, J=16.0Hz, 5.8Hz, 1H, -CH=CH-), 5.61 (s, 1H, O-CH-O), 5.53 (s, 1H, O-CH-O), 4.87(dd, J=5.8Hz, 1.5Hz, -CH-COO-).

(R)-5-(1'-Methyl-2'-phenylethenyl)-1,3-dioxolanone-4 derivative of 5 was obtained with 78% yield after recrystallization from hexane/$CHCl_3$ (1:2).

General procedure for the epoxidation of (R)-2-hydroxy-3-enoic acid derivatives with peracids. While stirring, 25 mmol peracid [m-chloroperbenzoic acid (MCPBA) or mono-perphthalic acid (MPPA)] in 20 ml hexane/diethyl ether (1:1) were dropped into a solution of 20 mmol (R)-2-hydroxy-3-enoic acid methylester in 30 ml diethyl ether. After stirring 3 days at room temperature, the starting material was not longer detectable by HPLC on a RP-18 column with 20% methanol in water eluent. The solution was washed with 3x20 ml 1N $NaHCO_3$ and finally with water. After the organic phase was dried with Na_2SO_4 and evaporated, an oily product was obtained, which crystallized slowly at 0°C.
The products formed were a mixture of two diastereomers, which could be separated on a cellulose triacetate column leading to the determination of de values. These were also confirmed by ^{1}H-NMR-analyses.
Characterization of the diastereomeric (R)-2-hydroxy-3,4-epoxy-4-phenyl-butanoic acid methylesters, obtained in a ratio of 73%:27% from MCPBA-epoxidation as an example: ^{1}H-NMR($CDCl_3$, AM-360): δ 7.26-7.37(m, 5H, arom.), 4.47 (d, 1H, -C(OH)H-), 4.1 (d, 73%x1H, Ph-CH-), 4.04(d, 27%x1H, Ph-CH-), 3.86 (s, 73%x3H, $COOCH_3$), 3.83 (s, 27%x3H, $COOCH_3$), 3.40 (dd, 73%x1H, -CH-C(OH)-), 3.37 (dd, 27%x1H, -CH-C(OH)-). ^{13}C-NMR($CDCl_3$, FX 90): δ 172.3, 147.68, 135.6, 128.4, 127.0, 125.8, 69.4(27%)+68.7(73%), 62.1(73%)+61.6(27%), 55.0, 53.0.
The epoxides of 5 methylester were obtained in a ratio of 53 : 47. Product with MCPBA: ^{1}H-NMR($CDCl_3$, WP-200): δ 7.33 (m, 5H, arom.), 4.29 (s, 53%x1H, -C(OH)H-), 4.15 (s, 46%x1H, -C(OH)H-), 4.18 (s, 46%x1H, Ph-CH-), 4.06 (s, 53%x1H, Ph-CH-), 3.92 (s, 46%x3H, $-COOCH_3$), 3.89 (s, 53%x3H, $-COOCH_3$), 1.08 (s, 3H, $-C(H)CH_3$-).

Conversion of 3,4-epoxy 1 methylester into lactone 7.
The 3,4-epoxy methylester 1 (0.5g) was dissolved in a mixture of 15 ml acetonitrile and 3 ml water, to which 1 ml 2N sulfuric acid was dropped and the homogenueous solution was stirred for 1 h. After evaporation of the solvent under vacuum, the residue was dissolved into 100 ml CH_2Cl_2, the solution washed with 20 ml water, 0.1 N $NaHCO_3$ and brine, and dried over Na_2SO_4. After filtration and evaporation, the residue of 385 mg crystalline product was recrystallized from chloroform. The first fraction was 185 mg 7 mp. 129-30°C. $[\alpha]_D$ = -88.5° (MeOH, 0.015 M). MS, M^+ 194. IR(KBr), 3580, 1768 cm^{-1} . ^{1}H-NMR (DMSO, AM-360): δ 4.0 (ddd, J_1= 8.5 Hz, J_2 = 8.9 Hz, J_3 = 6.1 Hz, H_{C3}); 4.4 (dd, J_1 = 8.9 Hz, J_2, = 7.1 Hz, H_{C2}); 5.0 (d, J= 8.5 Hz, H_{C4}); 6.1 (d, J=6.1 Hz, OH); 6.2 (d, J= 7.1 Hz, OH); 7.4 (m, 5H, arom.). NOE: Saturation of H_{C2} (4.40 ppm), H_{C4} (5.0 ppm) resulted in a positive signal, while H_{C3} gave none. Anal. calcd. for $C_{10}H_{10}O_4$ C: 61.91; H: 5.15; Found: C: 61.37; H: 5.12. NOE: Saturation of H_{C2} (4.4 ppm) resulted in a positive signal for H_{C4} and none for H_{C3}.
7b interpreted from the ^{1}H-NMR of the mixture with 7. δ 7.48 (m, 5H, arom.); 6.0 (d, OH); 5.79 (d, OH); 5.37 (d, J= 2.9 Hz, 1H); 4.30 (dd, 1H); 4.16 (m, 1H).

Hydroxylation of (R)-2-hydroxy-3-enoic acids and their derivatives
a. With $MeReO_3/H_2O_2$. Preparation of 7 and 7a was performed in analogy to l.c. [14]. Stirring 4 mg $MeReO_3$ in a solution of 6.3% hydrogen peroxide in 2 ml t-butanol for a few min. resulted in a yellowish solution, into which 2 mmol 1 methylester in 4 ml t-butanol were dropped. After stirring for 20 h at room temperature, HPLC (RP 18, 20% MeOH in water) showed the disappearance of the starting material and the formation of several products. The excess hydrogen peroxide was reduced by addition of 1 mmol $NaHSO_3$. After adding 1 ml 2N H_2SO_4 the solution was stirred for 2 h at room temperature and concentrated under vacuum. The residue was dissolved in 50 ml CH_2Cl_2, washed with 20 ml water and 20 ml 0.1 M $NaHCO_3$ and dried over Na_2SO_4. Evaporation of the solvent resulted in 350 mg (89%) crystalline product, which indicated a 7:3 mixture of two lactone diastereomers according to HPLC-analysis. After two recrystallizations of the mixture from chloroform, 165 mg (42%) (2R,3R,4S)-2,3-dihydroxy-4-phenyl-γ-butyrolactone 7 was obtained. This had the same ^1H- and ^{13}C-NMR spectra as the one obtained by epoxidation of 1 methylester with peracid.

b. With OsO_4/N-methylmorpholine-N-oxide (NMMO). In analogy to l.c.[5e] a solution of 192 mg (1 mmol) 1 methylester, 5 mg (0.02 mmol) OsO_4 and 156 mg (1.3 mmol) NMMO in 5 ml acetonitrile and 1 ml water was stirred for 20 h at room temperature. After addition of 50 mg $NaHSO_3$ and 10 min. stirring, 1 ml 2N H_2SO_4 and 2 ml acetonitrile were added and the solution was heated under reflux for 2 h. HPLC-analysis indicated a product mixture of 75:25. The solution was extracted three times with 3x15 ml CH_2Cl_2, the combined organic phases were washed with water, 0.1N $NaHCO_3$ and again with water and dried over Na_2SO_4. This led to 160 mg (7b+7c, 83%) crystalline residue which gave 80 mg 7b (41%) as white needles after recrystallization from chloroform. Mp. 87°C (decomp.); $[\alpha]_D$ = +42.1°(MeOH, 0.080 M); MS, M^+ 194, M(100) 107; ^1H-NMR(DMSO-d_6, AM-360): δ 4.36 (dd, J_1=4.4Hz, J_2=3.0Hz, 1H, H_{C3}), 4.59 (d, J=4.43Hz, 1H, H_{C2}), 5.1 (br, 2xOH), 5.5 (d, J=2.7Hz, 1H, H_{C4}), 7.3 (m, 5H, arom.); ^{13}C-NMR(DMSO-d6, AM-360): δ 176.2, 135.2, 127.8, 127.7, 126.9, 80.2, 71.7, 71.2; ^{13}C-NMR data of Beer et al.[15] (acetone-d_6, FX-90): δ 176.03, 135.93, 128.60, 127.69, 81.27, 72.8, 72.3. NOE: Saturation of any one proton of the lactone ring resulted in positive signals of the other protons.

(2R,3S,4S)-2,3-Di-O-isopropylidene-4-phenyl-γ-butyrolactone 7b'. One mmol lactone 7b (194 mg) obtained by dihydroxylation of 1 methylester with OsO_4 was dissolved with 20 mg p-toluene sulfonic acid in 10 ml dry acetone and refluxed for 3 h. The solution was concentrated under vacuum. Then the residue was dissolved in 20 ml diethyl ether and washed with 10 ml water, 10 ml 0.1N $NaHCO_3$ and 10 ml water. After drying with Na_2SO_4, filtration and evaporation of the diethyl ether, the solid residue was recrystallized from chloroform/ hexane (4:1) yielding 170 mg crystalline 7b', (70%), mp. 157-9°C. ^1H-NMR (acetonitrile-d_6, AM-360): δ 7.41 (m, 5H, arom.), 5.64 (d, J=2.85Hz, 1H), 5.0 (m, 2H), 1.37 (s, 3H), 1.28 (s, 3H); ^{13}C-NMR (acetonitrile-d_6, AM-360): δ 176, 129.5, 129.2, 127.7, 80.5, 79.4, 77.7, 27.1, 25.9.

Acetylated lactones 8b'/8c'. From 140 mg (1.1 mmol) 4 methylester, 160 mg (1.3 mmol) NMMO and 5 mg (0.02 mmol) OsO_4, 110 mg of the lactones were isolated as syrup with about 15% impurities after extraction with CH_2Cl_2 (3x15 ml) from the reaction solution and evaporation of the solvent to dryness. The syrup was then dissolved in 3 ml acetic anhydride and 3 ml pyridine and stirred at room temperature over night. The solution was concentrated under vacuum to a syrup and purified by preparative HPLC (RP-18, flow: 20 ml/min.; eluent: 20% MeOH in water) leading to 80 mg crystals (34%) consisting of a mixture of two lactones (60 : 40). MS: M+1 217; (2R,3S,4S)-2,3-di-O-acetyl-4-valerolactone 8b' (60%): ^1H-NMR($CDCl_3$, AM-360): δ 5.68 (d, J=5.0Hz, 1H, H_{C2}), 5.59 (dd, J_1=4.95Hz, J_2=3.1Hz, 1H, H_{C4}), 4.74 (dq, J_1=3.1Hz, J_2=6.5Hz, 1H, H_{C3}), 2.1 (2xCH_3, of acetyl groups), 1.42 (d, J=6.5Hz, 3H, CH_3(C-4)); ^{13}C-NMR ($CDCl_3$, AM-360): δ 170.19, 169.48, 169.21, 75.32, 71.0, 68.72, 20.46, 20.37, 14.08; (2R,3R,4R)-2,3-di-O-acetyl-4-valerolactone 8c' (40%): ^1H-NMR($CDCl_3$, AM-360): δ 5.50 (d, J=6.64Hz, 1H, H_{C2}), 5.46 (dd, J_1=6.62Hz, J_2=6.58Hz, 1H, H_{C3}), 4.98 (dq, J_1=J_2=6.6Hz, 1H, H_{C4}), 2.17 (2xCH_3 of the acetyl groups), 2.36 (d, J=6.7Hz, 3H, CH_3 at C-4).

Determination of diastereomeric excess without isolation of the products. For comparison, acid 1, 1,3-dioxolanone of 1 and 2-t-butyldimethylsilyl-ether of 1 methylester were hydroxylated on a 0.5 mmol scale with $MeReO_3$ or OsO_4, and after acid workup as described above, the two lactones were formed. They were assigned by HPLC comparing with the lactones 7/7a or 7b/7c. The de values were obtained by calculation of the peak areas.

Bromolactonization of (R)-2-hydroxy-3-enoic acids with N-bromo-succinimide (NBS) in N,N-dimethyl formamide (DMF)[21] (general procedure). The solution of 2 mmol (R)-2-hydroxy-3-enoic acid and 2.2 mmol NBS in 6 ml DMF was stirred at room temperature for 2 days, diluted with 50 ml diethyl ether and washed with water, 1N $NaHSO_3$, 0.1N $NaHCO_3$ and water, dried over Na_2SO_4 and filtrated. After evaporation of the solvents under reduced pressure, the crystalline residue was recrystallized from chloroform/hexane.

(2S,3R,4S)-2-Hydroxy-3-bromo-4-phenyl-γ-butyrolactone 9. Yield after recrystallization from hexane/chloroform (8:1), 55%, mp. 120-2°C; $[\alpha]_D$ = -21.9°(MeOH, 0.034M); MS: M^+ 256/258=1:1 indicates the bromine isotopes; IR(KBr): 3500, 1790cm^{-1}; ^{1}H-NMR($CDCl_3$, AM-360): δ 7.43 (m, 5H, arom.), 5.37 (d, J=9.6Hz, 1H, H_{C4}), 4.78 (d, J=10.1Hz, 1H, H_{C2}), 4.21 (dd, J_1=J_2=10Hz, 1H, H_{C3}), 3.95(br, OH); ^{13}C-NMR($CDCl_3$, AM-360): δ 173.16, 133.95, 129.90, 128.97, 126.72, 83.03, 75.87, 51.74; NOE: Saturation of the aromatic protons resulted in positive signals for H_{C3} (4.2 ppm) 6.2% and H_{C4} (5.4 ppm) 13.2%. Anal. calcd. for $C_{10}H_9BrO_3$: C, 46.71; H, 3.53; Found: C, 46.76; H, 3.50.

(2S,3R,4S)-2-Hydroxy-3-bromo-4-(p-chlorophenyl)-γ-butyrolactone 10. Yield after recrystzallization from hexane/chloroform (5:1), 60%, mp. 121°C; $[\alpha]_D$ = -45.7°(MeOH, 0.050 M); MS: M^+ 290/292/294 indicates the bromine and chlorine isotopes; IR(KBr): 3470, 1802 cm^{-1}; ^{1}H-NMR($CDCl_3$, AM-360): δ 7.4 (m, 5H, arom.), 5.35 (d, J=9.6Hz, 1H, H_{C4}), 4.79 (d, J=10.1Hz, 1H, H_{C2}), 4.16 (dd, J_1=J_2=10Hz, 1H, H_{C3}), 3.9 (br, OH); ^{13}C-NMR($CDCl_3$, AM-360): δ 172.9, 136.0, 132.5, 129.3, 128.0, 82.3, 75.7, 51.6; Anal. calcd. for $C_{10}H_8BrClO_3$: C, 41.20; H, 2.76; Found: C, 41.62; H, 2.79.

(2S,3R,4S)-2-Hydroxy-3-bromo-4-(2'-thienyl)-γ-butyrolactone 11. Yield after recrystallization from hexane/chloroform (4:1), 83%, mp. 106-110°C; $[\alpha]_D$ = -68.9°(MeOH, 0.035M); MS: M^+ 262/264=1:1 indicating the bromine isotopes; IR(chloroform): 3550, 1800cm^{-1}; ^{1}H-NMR($CDCl_3$, AM-360): δ 7.44 (d, J=5.1Hz, 1H), 7.26 (d, J=3.0Hz, 1H), 7.06 (dd, J_1=5.1Hz, J_2=3.0Hz, 1H), 5.62 (d, J=9.7Hz, 1H), 4.74 (d, J=10.1Hz), 4.33 (dd, J_1=9.7Hz, J_2=10.1Hz, 1H), 2.9 (br, OH); ^{13}C-NMR ($CDCl_3$, AM-360): δ 172.21, 135.88, 128.34, 127.85, 127.28, 79.04, 75.68, 51.28.

(2S,3R,4S)-2-Hydroxy-3-bromo-3-methyl-4-phenyl-γ-butyrolactone 12. Yield after recrystallization from hexane 71%; $[\alpha]_D$ = -18.3°(MeOH, 0.12M); MS: M^+ 270/272=1:1 indicates the bromine isotopes; IR(KBr) 3440, 1780cm^{-1}; ^{1}H-NMR($CDCl_3$, AM-360): δ 7.48 (m, 2H, arom.), 7.43 (m, 3H, arom.), 5.95 (s, 1H, H_{C4}), 5.77 (s, 1H, H_{C2}), 3.7 (br, OH), 1.34 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$, AM-360): δ 173.16, 131.52, 129.34, 128.47, 126.62, 85.18, 79.18, 63.26, 19.14. NOE: Saturation of H_{C2} resulted in positive signals of H_{C4} (5.95 ppm) 13%, H_{Me} (1.34 ppm) 2%, H_{arom} (7.45 ppm) 0%, saturation of H_{C4} (5.95 ppm) led to positive signals of H_{C2} (5.77 ppm) 13.5%, H_{Me} (1.34 ppm) 0.5%, H_{arom} (7.43 ppm) 8%.

(2S,3R,4S)-2-Hydroxy-3-bromo-4-(2'E-phenylethenyl)-γ-butyrolactone 13. Yield after recrystallization from chloroform 85% (reaction at 0°C); mp. 125-9°C; $[\alpha]_D$ = -37.0°($CHCl_3$, 0.070M); MS: M^+ 282/284=1:1, indicating the bromine isotopes; IR(KBr): 3464, 1765 cm^{-1}; ^{1}H-NMR(DMSO-d_6, AM-360): δ 7.43 (m, 2H, arom.), 7.35 (m, 3H, arom.), 6.84 (d, J=15.8Hz, 1H, Ph-C<u>H</u>=CH-), 6.11 (dd, J_1=15.8Hz, J_2=7.6Hz, 1H, Ph-CH=C<u>H</u>-), 5.0 (dd, J_1 =7.6Hz, J_2=9.7Hz, 1H, H_{C4}), 4.7 (d, J=10.0Hz, 1H, H_{C2}), 4.15 (dd, J_1=9.7Hz, J_2=10.0Hz, 1H, H_{C3}); ^{13}C-NMR(DMSO-d_6, AM-360): δ 173.3, 135.0, 128.8 (2xC), 127.1 (2xC), 121.1, 82.7, 75.4, 50.1.

Iodolactonization of (R)-2-hydroxy-3-enoic acids (General procedure). In analogy to l.c.[11] to a suspension of 2 mmol (R)-2-hydroxy-3-enoic acid in 10 ml water, 2.5 ml 1N KOH was added leading to about pH 12. Under stirring, 3 mmol I_2 and 4 mmol KI in 15 ml water were added. After 1 h further stirring at room temperature, 1.2 mmol $NaHSO_3$ was added. The solution was extracted with diethyl ether (2x20 ml), the water decanted, the brown residue remaining in the flask dissolved in 20 ml diethyl ether and combined with the ether extract obtained before. The ether solution was washed with water, 0.1N $NaHCO_3$, and brine, dried over Na_2SO_4 and filtrated. After evaporation of the solvent the residue was crystallized and recrystallized from chloroform/hexane.

(2S,3R,4S)-2-Hydroxy-3-iodo-4-phenyl-γ-butyrolactone 14. Yield after recrystallization from chloroform/hexane (1:1) 71%; mp. 137-8°C; MS: M+1=305; $[\alpha]_D$ = -24.2°(CH_2Cl_2, 0.043M); IR(KBr): 3500, 1788 cm^{-1}; ^{1}H-NMR ($CDCl_3$, AM-360): δ 7.54 (m, 2H, arom.), 7.49 (m, 3H, arom.), 5.80 (d, J=7.0Hz, OH), 5.60 (d, J=10.3Hz, 1H, H_{C4}), 4.86 (dd, J_1=7.0Hz, J_2=10.8Hz, 1H, H_{C2}), 4.33 (dd, J_1=J_2=10.6Hz, 1H, H_{C3}); ^{13}C-NMR($CDCl_3$, AM-360): δ 173.8, 136.7, 130.5, 129.8, 128.6, 84.1, 77.7, 28.9; NOE: Saturation of H_{arom} (7.41 ppm) resulted in H_{C2} (4.86 ppm) 0%, H_{C3} (4.33 ppm) 6.2%, H_{C4} (5.60 ppm) 13.2%. Anal. calcd. for $C_{10}H_9IO_3$: C, 39.45; H, 2.98; Found: C, 39.50; H, 2.93.

(2S,3R,4S)-2-Hydroxy-3-iodo-4-(p-chlorophenyl)-γ-butyrolactone 15. Yield after recrystallization from chloroform 58%; mp. 101-3°C; MS: M^+=338; $[\alpha]_D$ = -39.8°(MeOH, 0.034M); IR(KBr): 3380(br), 1775 cm^{-1}; ^{1}H-NMR (DMSO-d_6, AM-360): δ 7.40 (m, 4H, arom.), 5.45 (d, J=10.2Hz, 1H, H_{C4}), 4.77 (d, J=10.8Hz, 1H, H_{C2}), 4.12 (dd, J_1=10.2Hz, J_2=10.8Hz, 1H, H_{C3}), 2.16 (br, OH); ^{13}C-NMR(DMSO-d_6, AM-360): δ 173.58, 135.97, 132.52, 129.25, 128.36, 83.85, 77.03, 25.29.

(2S,3R,4S)-2-Hydroxy-3-iodo-4-(2'-thienyl)-γ-butyrolactone 16. Yield after recrystallization from chloroform 87%; mp. 121-3°C; MS: M^+=310; $[\alpha]_D$ = -82.2°(MeOH, 0.060 M); ^{1}H-

NMR(DMSO-d_6, AM-360): δ 7.43 (d, J=5.0Hz, 1H), 7.26 (d, J=2.3Hz, 1H), 7.06 (dd, J_1=5.0Hz, J_2=2.3Hz, 1H), 5.72 (d, J=10.3Hz, $1H_{C4}$), 4.77 (d, J=10.8Hz, 1H, H_{C2}), 4.32 (dd, J_1=10.3Hz, J_2=10.8Hz, 1H, H_{C3}), 2.6 (br, OH); ^{13}C-NMR(DMSO-d_6, AM-360): δ 173.04, 136.08, 128.69, 127.88, 127.28, 80.56, 25.39; Anal. calcd. for $C_8H_7IO_3S$: C, 30.98; H, 2.28; Found: C, 30.59; H, 2.30.

(2S,3R,4S)-2-Hydroxy-3-iodo-4-methyl-γ-butyrolactone 17. Yield after recrystallization from hexane/chloroform (4:1) 53%; mp. 143-7°C (lit.[11b] 115°C as racemic mixture); MS: M^+ 242; $[\alpha]_D$ = -26.6°(MeOH, 0.048M); IR(chloroform): 3400, 1780 cm^{-1}; ^{1}H-NMR($CDCl_3$, AM-360): δ 4.47 (dq, J_1=6.2Hz, J_2=10.3Hz, 1H, H_{C4}), 4.45 (d, J=10.7Hz, 1H, H_{C2}), 3.78 (dd, J_2=J_2=10.5Hz, 1H, H_{C3}), 1.36 (d, J=6.2Hz, 3H, CH_3); ^{13}C-NMR($CDCl_3$, AM-360): δ 173.24, 79.79, 77.05, 26.21, 17.71. NOE: Saturation of CH_3 protons (1.36 ppm) resulted H_{C2} (4.47 ppm) 0%, H_{C3} (3.78 ppm) 14%, H_{C4} (4.47 ppm) 45%.

Br_2-Addition to (R)-2-hydroxy-3-enoic acids or their derivatives. While stirring a solution of 1 g bromine dissolved in 10 ml CCl_4 was slowly dropped into a solution of 2 mmol (R)-2-hydroxy-3-enoic acid or its derivative in 25 ml CCl_4 at -20°C until the brown bromine color did not disappear quickly. The solution was then diluted with 20 ml diethyl ether, washed with 15 ml water, 15 ml 1N $NaHSO_3$, 15 ml 0.1N $NaHCO_3$ and brine, dried over Na_2SO_4 and filtrated. After evaporation of the solvent, the residue was crystallized from hexane/chloroform or water/ethanol.

(2S,3R,4S)-2-Hydroxy-3,4-dibromo-4-phenyl-butanoic acid 18. The Br_2 addition to 2 mmol 1 was carried out in CCl_4/diethyl ether (1:1) at -20°C. After evaporation of the solvent under vacuum the solid residue consisted of three products. After recrystallization from $CHCl_3$, the first fraction turned out to be pure 18 in a yield of 45%. Mp. 198-200°C; $[\alpha]_D$ = +60.1°($CHCl_3$, 0.070 M); MS: M^+ 336/338/340=1:2:1; IR(KBr): 3500, 1740 cm^{-1}; ^{1}H-NMR (DMSO-d_6, AM-360): δ 7.58 (d, 2H), 7.40 (m, 3H), 5.46 (d, 1H), 5.17 (dd, 1H), 4.98 (d, 1H); ^{13}C-NMR(DMSO-d_6, AM-360): δ 172.32, 139.90, 128.45, 128.36, 127.80, 70.64, 57.62, 53.50.

(2S,3R,4S)-2-Hydroxy-3,4-dibromo-4-phenyl-butanoic acid methylester 18'. The product was obtained first as a 95:5 mixture with near 100% yield. After recrystallization from water/ethanol (1:1), it was purified as 18' with 90% yield ; mp. 120°C; $[\alpha]_D$ = +50.7°($CHCl_3$, 0.069M); MS: M^+ 350:352:354= 1:2:1; IR($CHCl_3$): 3510, 1764 cm^{-1}; ^{1}H-NMR($CDCl_3$, AM-360): δ 7.36 (m, 5H), 5.32 (d, 1H), 5.16 (d, 1H), 4.89 (dd, 1H), 3.88 (s, 3H); ^{13}C-NMR ($CDCl_3$, AM-360): δ 172.04, 139.61, 129.04, 128.79, 127.90, 71.78, 57.50, 53.34, 52.52; Anal. calcd. for $C_{11}H_{12}Br_2O_3$: C, 37.52; H, 3.43; Found: C, 37.81; H, 3.80.

(2S)-5-(1',2'-dibromo-2'-phenylethenyl)-1,3-dioxolanone-4 was isolated as a diastereomeric mixture (80 : 20) in 82% yield. $[\alpha]_D$ = +33.9° as a mixture ($CHCl_3$, 0.068M); IR($CHCl_3$): 1800 cm^{-1}; ^{1}H-NMR($CDCl_3$, AM-360): δ 7.38 (m, 5H, arom.), 5.78 (s, 1H), 5.64 (s, 1H), 5.35 (d, J=1Hz, 1H), 5.22 (d, J=11.7Hz, 1H), 4.87 (dd, J_1=11.7Hz, J_2=1Hz, 1H); ^{13}C-NMR: δ 169.8, 138.9, 129.3, 128.9, 127.8, 96.3, 53.4, 74.9, 52.2.

(2S)-2-Hydroxy-3,4-dibromo-pentanoic acid methylester. A mixture of two diastereomers (75 : 25) was obtained in 87% yield. $[\alpha]_D$ of the mixture, = -18.0°($CHCl_3$, 0.069M); ^{1}H-NMR($CDCl_3$, AM-360): δ 4.97 (d, J=1Hz, 1H), 4.4 (m, 2H), 3.85 (s, 3H), 1.93 (d, 3H); ^{13}C-NMR($CDCl_3$, AM-360): δ 75%: 172.1, 73.7, 60.3, 53.28, 47.7, 25.9; 25%: 171.4, 72.1, 60.5, 53.14, 47.0, 26.0.

Hydrogenation of 5 isopropyl ester and methylester with $Rh(Ph_3P)_3Cl$. A mixture of 500 mg (0.086 mmol) tris-(triphenylphosphin)-rhodium chloride and 800 mg (3.4 mmol) 5 isopropylester in 15 ml dry CH_2Cl_2 was hydrogenated by shaking the solution under hydrogen gas at 35°C. The reaction was followed by both measuring the consumption of the hydrogen gas and HPLC of the reaction mixture with a RP-18 column (60% methanol in water). After three days, the starting material had disappeared. The solvent CH_2Cl_2 was evaporated and the residue was extracted with 4x20 ml hexane. After evaporation of hexane, 650 mg (82% yield) of an oily residue was identified by NMR analysis as a 85:15 diastereomeric mixture of 20 and 20a. Acid 5, its methylester and the 1,3-dioxolanone derivative were hydrogenated in a scale of 0.5 mmol. The product of the methylester was isolated as described for the isopropyl ester. The products of the two other hydrogenations experiments were analyzed for their diastereomeric ratio only.

Mixture of (2R,3R)- and (2R,3S)-2-hydroxy-3-methyl-4-phenyl-butanoic acid isopropylester (20 and 20a isopropylester). ^{1}H-NMR: δ 7.10-7.36 (m, 5H), 5.10 (hept., 1H), 4.06 (dd, 1H), 3.0 (d, 85%x1H), 2.88 (d, 15%x1H), 2.4-2.9 (m, 2H), 2.2-2.3 (m, 1H), 1.30 (d, 85%x6H), 1.25 (d, 15%x6H), 0.96 (d, 85%x3H), 0.81 (d, 15%x3H).

Mixture of 20 and 20a methylester. ^{1}H-NMR: δ 7.1-7.7 (m, 5H), 4.12 (dd, 1H), 3.73 (s, 18%x3H), 3.66 (s, 82%x3H), 2.70 & 2.80 (dd, 1H), 2.46 & 2.58 (dd, 1H), 2.27 (m, 1H), 0.96 (d, 82%x3H), 0.84 (d, 18%x3H).

Hydrolysis of the mixture of 20/20a isopropyl ester with esterase. A mixture of 0.6 g ester (85:15) and 0.25 ml esterase solution (Sigma, EC 3.1.1.1., 260 units/mg protein from bovine liver) in 60 ml 0.1M potassium phosphate buffer pH 8.0 was stirred for 48 h at room temperature. The solution was extracted by diethyl ether (2x30 ml), and the aqueous phase was acidified with sulfuric acid to pH 2.0. This solution was again extracted with diethyl ether. After drying the ether phase with Na_2SO_4 and evaporation, 0.2g oily residue was identified as acid **20/20a** (85:15) by HPLC analyses. That means, they are identical with the hydrogenation product of acid **5**.

Pechmann degradation of the 85:15 mixture 20/20a to 21/21a. An emulsion of 80 mg **20/20a** and 0.7 g $Ce(SO_4)_2$ in 25 ml, 0.3N sulfuric acid was heated at 90°C for 10 h and the solution extracted with diethyl ether (3x20 ml). As seen by HPLC the 50 mg oily residue of the ether phase was a mixture of 70% 2-methyl-3-phenyl-propionic acid **21/21a** (85:15) and 30% benzoic acid. **21/21a** were purified by preparative HPLC.

Synthesis of racemic and (R)-2-methyl-3-phenyl-propionic acid. For comparison, the racemic 2-methyl-3-phenyl-propionic acid was obtained by hydrogenating the 2-methyl-3-phenyl-2-propenoic acid with Pd/C as catalyst. (R)-2-Methyl-3-phenyl-propionic acid **21** was prepared by hydrogenating 2-methyl-3-phenyl-2-propenoic acid with enoate reductase from *Clostridium tyrobutyricum*.[20]

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REFERENCES

1. (a) Simon, H.; Bader, J.; Günther, H.; Neumann, S. W.; Thanos, *J. Angew. Chem. Int. Ed. Engl. 24*, 539-553. *Angew. Chem.*, **1985**, *97*, 541-55. (b) Neumann, S. W. *Ph. D. Thesis*, Technical University of Munich, **1985**. (c) Günther, H.; Neumann, S. W.; Simon, H. *J. Biotechnol.*, **1987**, *5*, 53-65. (d) Skopan, H.; Günther, H.; Simon, H. *Angew. Chem. Int. Ed. Engl.*, **1987**, *26*, 128-30. (e) Thanos, J.; Bader, J.; Günther, H.; Neumann, S. W.; Krauss, F.; Simon, H. *Methods in Enzymology*, **1987**, *136*, 302-17. (f) Simon, H. in *Biocatalysis*, Abramowicz, D.A. Ed.; van Nostrand Reinhold: New York, p. 217-242, **1990**.

2. (a) Schummer, A.; Yu, H.; Rauschenbach, P.; Schinschel, C.; Simon, H. in: *DECHEMA Biotechnology Conferences*, 3. Ed., D. Behrens, VCH Weinheim, p271, **1989**. (b) Schummer, A.; Yu, H.; Simon, H. See preceeding paper in this journal.

3. (a) Bartlett, P. A. *Tetrahedron*, **1980**, *36*, 3-74. (b) Berti, G. in E.L. Eliel & N.L. Allinger Ed. *Topics in Stereochemistry*, Wiley-interscience, New York, **1973**, *7*, 93-251. (c) Rebek, J. Jr. *Heterocycles*, **1981**, *15*, 517-45. (d) Bartok, M.; Lang, K. L. *Heterocyclic Compounds*, **1980**, *10*, 1-196.

4. (a) Sharpless, K. B.; Verhoeven, T. R. *Aldrichimica Acta*, **1979**, *12*, 63-73. (b) Sharpless, K. B.; Woodard, S. S.; Finn, M. G. *Selectivity, a Goal for Synthetic Efficiency*, **1988**, 376-89. (c)Gao, Y.; Hanson R.M.; Klunder, J. M.; Ko, S-Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.*, **1987**, *109*, 5765-80. (d) Wang, Z-M.; Zhou, W-S., *Tetrahedron*, **1989**, *43*, 2935-44.

5. (a) Cha, J. K.; Christ, W. J.; Kishi, Y. *Tetrahedron Lett.*, **1983**, *24*, 3943-6. (b) ibid, 3947-50. (c) Jacobsen, E. N.; Marko, I.; Mungall, W. S.; Schröder, G.; Sharpless, K. B. *J. Am. Chem. Soc.*, **1988**, *110*, 1968-70. (d) Wai, J. S. M.; Marko, I.; Svendsen, J. S.; Finn, M. G.; Jacobsen, E. N., Sharpless, K. B. *J. Am. Chem Soc.*, **1989**, *111*, 1123-5. (e)VanRheenen, V.; Kelly, R. C.; Cha, Y. *Tetrahedron Lett.* **1976**, *17*, 1973-6.

6. (a) Van Tamelen, E. E.; Shamma, M. *J. Am. Chem. Soc.*, **1954**, *76*, 2315-7. (b) Barnett, W. E.; Sohn, W. H. *Tetrahedron Lett.*, **1972**, *13*, 1777-80. (c) Cambie, R. C.; Hayward, R. C.; Roberts, J. L.; Rutledge, P. S. *J. Chem. Soc., Perkin I*, **1974**, 1864. (d) Bartlett, P. A.; Richardson, D. P.; Myerson, J. *Tetrahedron*, **1984**, *40*, 2317-27. (e) Chamberlin, A. R.; Dezube, M.; Dussault, P. *Tetrahedron Lett.*, **1981**, *22*, 4611-4. (g) Terashima, S.; Jew, S.S. *Tetrahedron Lett.*, **1977**, *18*, 1005-8. (h) Fuji, K.; Node, M.; Naniwa, Y. & Kawabata, T. *Tetrahedron Lett.*, **1990**, *31*, 3175-8.

7. Labelle, M.; Guindon, Y. *J. Am. Chem. Soc.* **1989**, *111*, 2204-10.

8. (a) Santalli, M.; Viala, J.; Pioncare, R. H. *Tetrahedron Lett.* **1977**, *18*, 4397-9. (b) Ganem, B. *J. Am. Chem. Soc.* **1976**, *98*, 858-9.

9. (a) Brown, J. M. *Angew. Chem.*, **1987**, *99*, 169-84. (b) ApSimon, J. W.; Lee Collier, T. *Tetrahedron*, **1986**, *42*, 5157-254. (c) Evans, D. A.; Morrissey, M. M.; Dow, R. L. *Tetrahedron Lett.* **1985**, *26*, 6005-8.

10. Stork, G.; Kahne, D. E. *J. Am. Chem. Soc.* **1983**, *105*, 1071-3.

11. The epoxidation of and iodine addition to the racemic 2-hydroxy-3-pentenoate were performed. (a) Sasaki, S.; Ito, M.; Fugiso, M. *Nippon Kagaku-Zasshi*, **1963**, *84*, 351-3. (b) Rossi, A.; Schinz, H. *Helv. Chim. Acta*, **1948**, *31*, 473-92.

12. This assumption is based on the ^{1}H-NMR spectrum of the product mixture. A double quartet at 4.8 ppm is characteristic for the lactone proton at the position C-4.

13. (a) Seebach, D.; Naef, R.; Calderari, G. *Tetrahedron*, **1984**, *40*, 1313-24. (b) Polt, R.; Seebach, D. *J. Am. Chem. Soc.*, **1989**, *111*, 2622-32.

14. (a) Herrmann, W.H.*J. Organomet. Chem.* **1990**,*382*,1-18. (b) Marz, D. *Ph. D. Thesis*, Technical University of Munich, **1990**.

15. Beer, D.; Meuwly, R.; Vasella, A. *Helv. Chim. Acta*, **1982**, *65*, 2570-82.

16. With lactone **14** as an example, the sequence of the groups attached to the chiral center are defined as:C-2, -OH > -CHI-C > -COO-; C-3, I > -C(Ph)-O-C- > -CH(OH)COO- >; C-4, -O- > CHI- > -Ph-.

17. Rolston, J. H.; Yates, K. *J. Am. Chem. Soc.* **1969**, *91*, 1469-77.

18. Olah, G. A.; Schilling, P.; Westerman, P. W.; Lin, H. C. *J. Am. Chem. Soc.* **1974**, **96**, 3581-3589.

19. Simon, H.; Floss, H. G. *Anwendung von Isotopen in der Organischen Chemie und Biochemie*, Band I, Springer-Verlag, Berlin, **1967**, 48-50.

20. (a) Leinberger, R.; Hull, W. E.; Simon, H.; Retey, J. *Eur. J. Biochem. 1981*, 117, 311-318. (b) Görgen, G.; Boland, W.; Preiss, U.; Simon, H. *Helv. Chim. Acta 1989* 917-928. (c) Preiss, U. *Ph. D. Thesis 1990*, Technical University Munich.

21. This is a modified procedure as described in ref. **6g**.