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Polyfunctional (R)-2-Hydroxycarboxylic Acids by Reduction of 2-Oxo Acids with Hydrogen Gas or Formate and Resting Cells of *Proteus vulgaris*

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Abstract: Various (R)-2-hydroxy acids such as (R)-2-hydroxy-3-enoic-, 3,5-dienoic-, 4-oxo-, (R,S)-3-hydroxy and some others were prepared on a scale up to 0.12 mol by biocatalytic reduction of the corresponding 2-oxo acids with *P. vulgaris* and hydrogen gas and/or formate as electron donors. With the exception of the 2-hydroxy-4-oxo acids it could be proved that the enantiomeric excess is >97%. For the 4-oxo derivatives this enantiomeric excess can be assumed. The yields of isolated products are high because they were isolated from rather small amounts of biocatalyst and low buffer concentrations. Product concentrations in the range of 0.1-0.24 M were obtained. For 1 mmol of product formation in 15-20 h about 20-40 mg (dry weight) of *P. vulgaris* cells are necessary.

Introduction

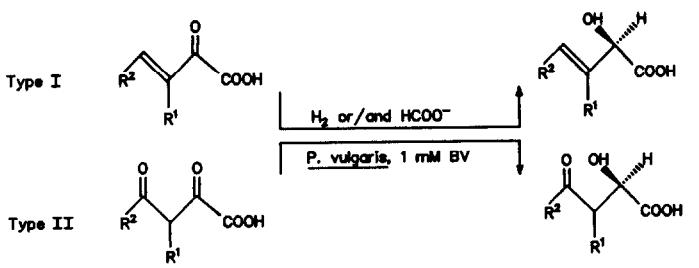
Chiral 2-hydroxy carboxylic acids are valuable synthons.[1] This is especially true if they contain additional functional groups which by chiral induction can be converted to further chiral centers in diastereoselective chemical reactions. From this point of view chiral 2-hydroxy-3-enoic- or 2-hydroxy-4-oxo carboxylates, as shown in the Scheme, should be of interest. So far such compounds hardly have been described and chiral chemical reductions seem to lack the necessary chemoselectivity for 2-oxo-3-enoic or 2,4-dioxo carboxylates.

Already some time ago we showed that resting cells of *Proteus vulgaris* are exceptionally efficient in reducing structurally very different 2-oxo acids to (R)-2-hydroxy acids in high yields and with complete stereoselectivity.[2] According to the Scheme hydrogen gas or formate can be used as reducing agents for the 2-oxo acids. This is possible since *P. vulgaris* possesses in addition to a 2-hydroxy carboxylate viologen oxido reductase (HVOR) a hydrogenase and a viologen-dependent formate dehydrogenase. The HVOR is able to accept electrons from reduced viologens as well as to deliver electrons to oxidized viologens and a series of other artificial electron acceptors.[3a,b] The HVOR detected by us a few years ago is a new type of a molybdenum, iron/sulfur containing redox enzyme which does not react with pyridine nucleotides.[2c] Information on properties of the purified enzyme which is membrane bound in the cells will be described elsewhere. The reactions displayed in the Scheme do not mean that a fermen-

tation is taking place, which usually means low concentrations of product and difficult isolation. From a practical point of view wet packed or freeze dried cells of *P. vulgaris* are a rather stable biocatalyst possessing not only an enzyme for reducing many different 2-oxo carboxylates but also the enzymes for the regeneration of the reduced form of the artificial electron mediators. Besides viologens, usually applied in 1 mM concentrations, complexes such as cobalt sepulchrate can be used.[2c] The product concentration is usually 100-220 mM.
We reduced a series of 2-oxo-3-enoic-, 2-oxo-3,5-dienoic- and 2,4-dioxocarboxylates as well as some racemates with a chiral carbon atom in 3-position to the corresponding (R)-2-hydroxy carboxylates. The scale varied up to 120 mmol. Especially 2-oxo-4-phenyl-3-enoate (1) but also a few others were used to optimize the reduction and to prove that preparative reactions can be easily conducted in a normal organic laboratory. Furthermore, a few more commercially available 2-oxo carboxylates were converted giving additional examples for the broad substrate specificity and applicability of this biocatalyst.
We showed that the hydroxy enoates can be converted to many different synthons with 2 or 3 chiral centers as the result of a two step reaction sequence starting from the 2-oxo acid.[4a,b]

Results and Discussion

Preparation of 2-oxo acids and racemic 2-hydroxy carboxylates. A detailed review article[5] desribes 19 methods for the preparation of 2-oxo acids. The type of 2-oxo-3-enoic acids is mentioned only briefly. We prepared 2-oxo carboxylates of Type I and II and a group of miscellaneous compounds and reduced them stereospecifically according to the following Scheme:



Scheme. Types of reduced 2-oxo- acids.

R^1 and R^2 are shown in Table 1.
For R of the miscellaneous forms of R-CO-COOH see Table 3.

The different 2-oxo acids were prepared by several routes according to reactions (1-4).

$$R^2CHO + R^1CH_2COCOOH \longrightarrow R^2CH{=}CR^1COCOOH \quad (1)$$
$$R^2CH{=}CR^1\text{-}MgBr + EtOOCCOOEt \longrightarrow R^2CH{=}CR^1COCOOEt \quad (2)$$
$$R^2COCH_2R^1 + EtOOCCOOEt \longrightarrow R^2COCHR^1COCOOEt \quad (3)$$
$$RCHCl\text{-}COCOOR \longrightarrow RCHOHCOCOOR \quad (4)$$

Table 1. 2-Oxo carboxylates of type I and II synthesized according to eq 1-4. (For the position of R^1 and R^2 see Scheme).

Compound	R^1	R^2	Prepared by method	Yield (%)
type I				
1	H	C_6H_5	1	80
2	Me	C_6H_5	1	89
3	H	p-ClC_6H_4	1	43
4	H	p-BrC_6H_4	1	65
5	H	2-thienyl	1	62
6	H	2-furyl	1	70
7	H	methyl	2	58
8	H	Ph-CH=CH	1	89
9	Me	Ph-CH=C	1	52
type II				
10	H	Ph	3	44
11	H	Ph	3	21
12	-$(CH_2)_4$-		3	42

2-Oxo-acids (**1-6, 8, 9**) (Table 1) were obtained in analogy to Reimer and others[6] by condensing aromatic aldehydes or derivatives such as cinnamic aldehyde with pyruvate or 2-oxobutyrate (eq. 1). A rather general method based on work of Rambaud et al.[7] seems to be the condensation of the Grignard products of 1-bromo-1-alkenes with diethyl oxalate (eq. 2). The 2,4-dioxo carboxylates (Type II) were prepared from suitable ketones and diethyl oxalate (eq.3)[8] and the 2-oxo-3-hydroxy acids such as **17** by hydrolyzing the corresponding ethyl 3-chloro-2-oxo-carboxylates (eq.4) which in turn were prepared from epoxides formed by condensing aldehydes with ethyl dichloroacetates.[9] Table 1 shows the 2-oxo acids prepared by eqs. 1-3. Other 2-oxo acids were obtained from commercial sources.

The yields of type I oxo acids were between 43 and 89%. Those for the 2,4-dioxo carboxylic acids were lower (Table 1). Only in a few cases the preparation of the 2-oxo acids was optimized.

If esters were the products of the 2-oxo acid synthesis the hydrolysis was conducted as a separate step by alkaline hydrolysis or in the case of labile compounds by Lipozyme^R at neutral pH. Especially unstable carboxylates such as the 2,4-dioxocarboxylates **10, 11** and **12** were formed enzymatically by Lipozyme^R from the esters during the bioreduction. Esters of 2-oxo acids are not converted by the HVOR. If ethyl 2,4-dioxocarboxylates are used as starting material for the bioreduction the ratio of Lipozyme^R and *P. vulgaris* has to be optimized. If too little lipase is used, the reaction proceeds very slowly. If too much lipase is used, the fast hydrolysis leads to the labile carboxylate for which it takes some time until it is reduced with the consequence of side reactions.

The racemic mixtures of the 2-hydroxy-3-enoic acids were prepared by reduction of the oxo acids with sodium borohydride in the presence of equimolar concentrations of cer trichloride in analogy to Luche.[10] Ce-III inhibits the reduction of the carbon carbon double bond adjacent to the car-

bonyl group. This method did not work for the regioselective reduction of 2,4-dioxo-carboxylates or esters.

The biocatalyst. *Proteus vulgaris* cells were grown on scales between 1 and 300 liters[3a] and stored as wet packed cells under the exclusion of oxygen at about -15° C. Freeze dried cells, again prepared under the exclusion of oxygen, can be stored at room temperature. Under both conditions the loss of reduction activity is less than 10% during 18 months of storage.[2c] The capability of reduction of the cells is expressed by the so called productivity number which is defined as follows:[2a]
PN = mmol product formed per kg of dry weight biocatalyst and hour.

Reduction procedures. The reductions were conducted on scales from 0.3 up to 120 mmol in the presence of 1 mM benzylviologen. Usually 100 mg wet packed cells, corresponding to 20 mg dry weight, were used for the reduction of 0.3 mmol 2-oxo acid in 3 ml at the expense of hydrogen gas in order to compare various substrates or to study parameters such as pH dependence etc. The substrate concentration was mostly 0.1 M. To obtain higher product concentrations favorably on a preparative scale substrate can be added repeatedly after most of the original 0.1 M substrate is reduced.
The preparative bioreductions were conducted by various protocolls: (i) With hydrogen gas under atmospheric pressure, (ii) with sodium formate under an atmosphere of nitrogen and (iii) by the combination of hydrogen gas and sodium formate.
In order to judge the dependence of reduction rates on various factors one has to be aware of the fact, that the reduction of a 2-oxo acid according to the Scheme consists of a series of consecutive reactions.
First the oxidized viologen is reduced by hydrogenase or formate dehydrogenase at the expense of hydrogen gas or formate.

$$2V^{++} + H_2 \quad (\text{or } HCOO^-) \longrightarrow 2V^{+\cdot} + 2H^+ \quad (\text{or } CO_2 + H^+) \quad (5)$$

In the second reaction the reduced viologen transfers electrons to the HVOR which in turn reduces the 2-oxo acid. Usually the first reaction is rate limiting.
If 1 mM benzylviologen or another viologen is used as a mediator the reaction mixture is not or only faintly blue until the reaction is almost completed. Then it turns to an intensive blue color, indicating that the reduction of the viologen is faster than the consumption of the reduced form.
The use of hydrogen gas for the reduction has several advantages. The progress of the reaction can be followed by the hydrogen consumption and the pH value of the reaction solution does not change during the reduction. One molecule of dihydrogen delivers two electrons and two protons necessary for the reduction. However, hydrogen gas under normal pressure is less effective than formate and the larger the volume of the liquid phase the more difficult it is to saturate the liquid phase with hydrogen gas, if no increased pressure is applied. The use of formate also has several advantages. A homogeneous system can easily be enlarged. The reaction rate does not depend on shaking frequency or stirring rate as it is the case with

hydrogen gas as electron donor. Gentle stirring is sufficient if formate is used. It is more convenient to handle sodium formate than hydrogen gas. Disadvantages of the use of formate are that the progress of the reaction cannot be observed directly and that for the reduction of an oxo acid with formate an additional proton is necessary leading to an increase of the pH value during the reaction. The use of high buffer concentrations increases cost and means less elegant product isolation. Therefore, an automatic pH control system was applied which added formic acid during the reaction. Under these optimized conditions phosphate buffer concentrations of 0.02 M and 20 mg cells of *P. vulgaris* (dry weight) for the reduction of 1 mmol substrate in 0.1 M concentration were used.

The aforementioned optimizations for preparative conversions were conducted with substrate **1**. The rate dependence on pH shown in Fig. 1 reveals a rather clear optimum at pH 7. Fig. 2 shows the time course of reduction of **1** with hydrogen gas, formate or the combination of both reducing agents. The differences in rates are not always as large as in the example depicted here. But as can be seen from Tables 2 and 3 formate always causes a faster reduction than hydrogen gas. In the region of substrate reduction up to 80% the combination of hydrogen gas and formate leads to higher reduction rates than the sum of the rates observed with a single reducing agent. That is demonstrated by measurements shown in Table 4 and was also observed in other cases.[2b] The rate of reduction expressed in productivity numbers (PN) in the presence of formate and hydrogen gas after 50 and 80% conversion is twice that of the sum of the reaction rate observed with hydrogen gas and formate only. Furthermore, Fig. 2 shows that the reduction of the last 5 - 10% of the substrate proceeds relatively fast. That is the consequence of low K_m values (Table 5) and low inhibition by the product.

The reduction shows a rather pronounced substrate inhibition by **1** in the region from 0.1 to 0.2 M (Table 4). In the presence of hydrogen gas and a 0.2 M solution of **1** the rate is diminished resulting in a PN 64% of that for a 0.1 M solution. When formate is applied as reducing agent the PN for a 0.2 M solution of **1** is only 10% of that of a 0.1 M solution. This indicates that the formate dehydrogenase is more inhibited by increased 2-oxo acid concentrations than the hydrogenase. Fig. 3 shows the time course of the reduction of **1** if the substrate is added in portions to a proceeding reaction and gives a protocoll if high PNs, high product and low buffer concentrations are aimed at.

As can be seen from Tables 2 and 3 most of the 20 different 2-oxo acids can be reduced with PNs in the region from 6 000 to more than 30 000. The conditions were not optimized in all cases. The indicated yields correspond to the isolated material if more than 0.3 mmol substrate was converted. The product isolation is rather simple since relatively low amounts of biocatalyst are necessary and the conversion of substrate is complete.

In general there is no drastic effect of the residue adjacent to the 2-oxo group on the rate of reduction. The rates are all in the range of one order of magnitude. One or two carbon carbon double bonds conjugated to the 2-oxo group are tolerated (products **1a-9a**). An additional oxo group in 4-position is not reduced (products **10-12**). Carbon atom 3 may be secondary, tertiary or quarternary. In the latter case (**20**) the reduction rate is re-

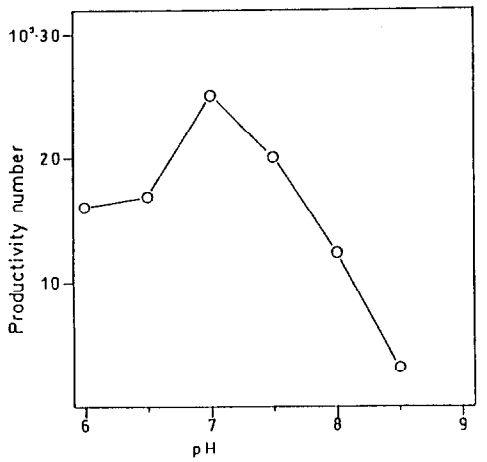


Figure 1. pH-Dependence of the reduction of **1** by *P. vulgaris* in the presence of hydrogen gas. For pH 6.0 - 8.0 potassium phosphate buffer and for 8.0 - 8.5 Tris buffer was applied.

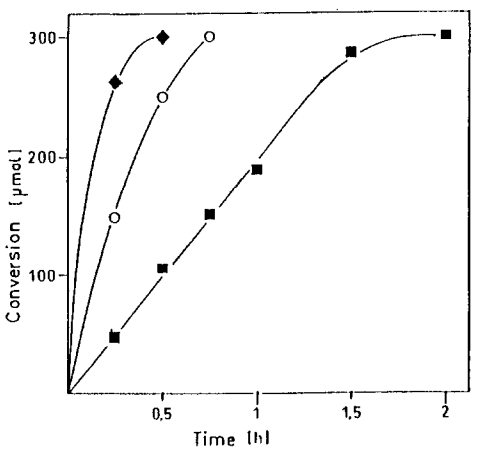


Figure 2. Reaction progress for the reduction of **1** with *P. vulgaris* at the expense of 1 atm of hydrogen gas ■—■, formate O—O and the combination of hydrogen gas and formate ◆—◆.

latively low (Table 3). Also fluorine or hydroxy groups at carbon atom 3 are accepted. However, as observed with 3-bromopyruvate and 2-oxo-3-chloro-4-methyl pentanoate chlorine as well as bromine in 3-position lead to an inhibition of the HVOR and products cannot be detected (not shown).

On the basis of the broad substrate specificity of HVOR it is not surprising that the two enantiomers of oxo acids with a chiral center at carbon atom 3 are reduced with similar rates as shown in Fig. 4. The time course of the reduction of 0.01 mol of **17** with hydrogen gas as found by taking small aliquot parts and analyzing them by reverse phase HPLC shows after 19% conversion a de = 69%. At the end of the reaction both diastereomers were present in equal amounts. The course of reduction of (R,S)-2-oxo-3-methyl-pentanoate (**18**) was similar to that of the 3-hydroxy compound (**17**). After 32% reduction a diastereomeric excess of about 60% was observed. Even rather large residues at C-3 do not change this picture. Both enantiomers of **11** showed at first again differences in rates as in the two former cases. The isolated product **11a** was, according to HPLC in an aqueous methanol eluent, a 1:1 mixture of two diastereomers. As seen by

Table 2. (R)-2-Hydroxycarboxylates obtained by reduction of 2-oxocarboxylates of type I and II (see Table 1) with *P. vulgaris* and various reducing agents in the presence of 1 mM benzylviologen. If not mentioned otherwise the ee was >97%. PN_{80} gives the productivity number after 80% product formation.

Compound	Scale [mmol]	Reducing agent	PN_{80}	Yield[a] [%]
1a	100	formate	17 800	> 90
1a	0.3	H_2	9 800	
2a	0.3	H_2	8 900	
2a	10-100	formate	11 700	87
3a	10	H_2	9 200	96
4a	10	H_2	7 600	90
5a	10	H_2	11 000	90
5a	10	formate	31 000	90
6a	10	H_2	13 800	78
7a	10-120	H_2	11 500	70-90
8a	10	H_2	8 300	98
9a	10	H_2	4 000	95
10a	10-45	formate	-[b]	87
11a	2	formate	-[b]	44
12a	10	formate	-[b]	74[c]

[a] Isolated material; [b] The carboxylates were formed during the reaction from ethyl esters by Lipozyme[R]. The hydrolysis was rate limiting. PN of 1 000 - 3 000 were usually observed. Chromatography on a Chiral-1 column showed one peak only. However, racemates of **10a-12a** were not available. Therefore it could not be proved that also the racemates of **10, 11** and **12** are separated on a Chiral-1 column. [c] Yield determind by weight of the residue of ether extract.

NMR analysis in $CDCl_3$ a diastereomeric ratio of 1:9 exists. This is probably due to enolization. Only the enol form of the oxo acid **11** could be observed in chloroform by ^1H- and ^{13}C-NMR.

In cases in which only the esters of a 2-oxo acid are stable the acids can be generated *in situ* during the reaction at neutral pH using a lipase or esterase. This was shown for the substrates **10-12**. In these cases the rate of reduction was about 20-30% of **1** because the enzymatic hydrolysis was rate limiting.

2-[^{2}H]-(R)-2-hydroxy acids. *P. vulgaris* can also be used in ^2H_2O for the reduction of 2-oxo acids. This was shown with carefully freeze dried cells which were suspended in buffer made from 99.9% ^2H_2O. The reduction rate of **1** at p^{2}H 7.0 is about 25% of the rate in normal water. The system was not optimized. Due to a solvent isotope effect the rate could be considerably higher at another p^{2}H value. By sensitive NMR spectroscopy no proton could be detected in 2-position of **1a** when it was prepared in ^2H_2O-buffer. The yield of the pure isolated product was 95%.

Kinetic studies. For some of the above mentioned new substrates the appa-

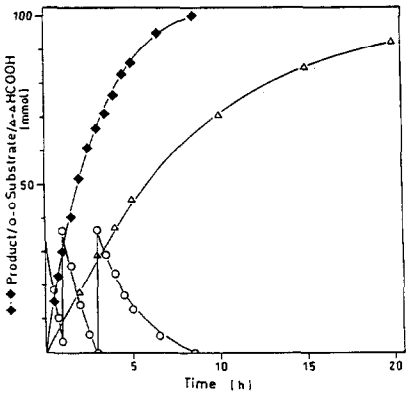


Figure 3. Time course of the reduction of altogether 0.1 mol **1** in a final volume of 450 ml 0.02 M potassium phosphate buffer, pH 7.0 by 9.2 g wet packed cells (= 1.8 g dry weight) of *P. vulgaris*, 1 mM benzylviologen at the expense of formate. Start: 0.033 mol **1** and 0.025 mol formate after about 1.5 and 3 h additional 0.033 mol **1** were added. The ph was kept at 7.0 by a pH-control system which added formic acid to the system.

Table 3. Further (R)-2-hydroxy acids R-CHOH-COOH obtained from the corressponding 2-oxo acids (RCOCOOH) by *P. vulgaris* catalyzed reduction. Products obtained from 0.3 mmol substrate were not isolated. By HPLC it was shown that all the substrate was consumed leading to a chiral product as indicated by chromatography on a Chiral I column.

Compound	R	Scale [mmol]	Reducing agent	PN_{80}[a]
13a	C_2H_5	0.3	H_2	10 000
14a	$CH_3(CH_2)_5$	10-45	formate	26-35 000[a]
15a	FCH_2	0.3	H_2	7 000
16a	$HOCH_2$	0.3	H_2	8 550
17a	(R,S)-n-C_3H_7-CHOH[b]	10	H_2	6 250
18a	(R,S)-$C_2H_5CHCH_3$	0.3	H_2	8 450
19a	Me_2CH	0.3	H_2	10 000
20a	Me_3C	0.3	H_2	2 400

a) Sometimes the conversion of the last 20% of product occurred over night without observing the rate. Therefore, the productivity number after 80% product formation is given. b) Synthesized according to eq. (4)

rent overall K_m and V_{max} values were determined by spectrophotometric methods as described.[3a] The enzyme source was a crude extract of *P. vulgaris* cells. Phenylpyruvate was used as a reference substance (Table 5). As long as there is no branching in ß-position the K_m values are not very different. A methyl group in ß-position increases the K_m by a factor of about 3 compared with the corresponding unbranched compound. The value for 2-oxo-3,3-dimethylbutanoate is 7.5 mM as already determined earlier.[3a] That means, that a second methyl group increases the K_m-value by another factor of 3. An electron withdrawing substituent such as an OH group or a fluorine atom diminishes the reaction rate by a factor of 3 and about 7, respectively.

Determination of the stereochemical purity and proof of regioselectivity. *P. vulgaris* exclusively forms the R enantiomer as already shown for a se-

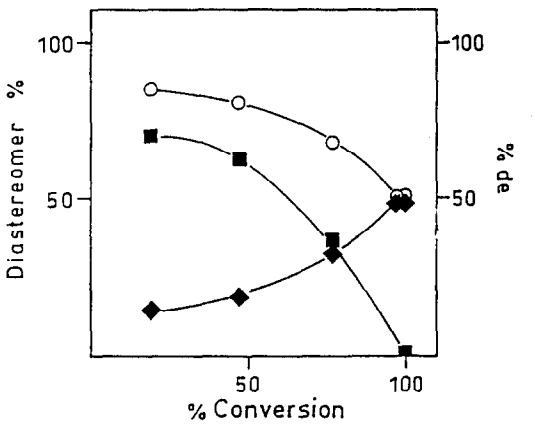


Figure 4. Course of the reduction of racemic **17** ■—■ and relative amounts of two diastereomers o—o and ◆—◆.

Table 4. Productivity numbers (PN) observed for the reduction of **1** in a concentration of 0.1 and 0.2 M using hydrogen gas, formate or their combination. PN for 0.2 M solutions are given in parenthesis.

Electron Donor	PN after Product Formation of 50	80	100%
H_2	10 000 (6 400)	9 800	8 600
$HCOO^-$	30 000 (3 000)	25 900	20 000
H_2 + $HCOO^-$	91 000 (10 000)	68 600	30 000

ries of other 2-hydroxy carboxylates obtaind from 2-oxo acids by various methods [2a-d]. All of the 2-hydroxy carboxylates prepared here were analyzed on a chiral HPLC column by ligand exchange chromatography using a dilute copper sulfate solution as eluent. As already shown α values for both enantiomers of aromatic as well as for aliphatic 2-hydroxy acids are in the range of 1.3-1.8 with higher retention times for the (R)-2-hydroxy acids.[11] We observed values of 1.17 - 1.6 (Table 6). This method was applied by us to more than 10 different hydroxy acids. In all cases using the column Chiral-1 the R- and S-enantiomer are well separated. Seven examples are shown in Table 6. In six other cases it was indepentendly shown by us[2c-d] that the R-enantiomer has the higher retention volume. This is in accordance to the literature.[11] Therefore, we assume that this is also the case for such 2-hydroxy acids for which no independent determination was conducted. Assignments of a single enantiomer based on the retention time only should not be made, since the retention time of a 2-hydroxy acid depends under otherwise completely identical conditions on amount of acid applied to the column. All hydroxy acids mentioned in Tables 2 and 3 gave only one peak by this analytical procedure. By adding defined small amounts of the corresponding racemate in the cases of **1a - 6a** we came to the conclusion that the ee values are at least >97% for all Type I hydroxy acids and for such mentioned in Table 3. However, for the products of type II, due to lack of regioselectivity of the usual reducing agents for oxo groups, we did not prepare the racemic 2-hydroxy-

Table 5. K_m and V_{max} Values of some 2-oxo acids as determined with cell free extract of *P. vulgaris* at 25° C. Phenylpyruvate was used as a standard.

Substrate	K_m (mM)	V_{max} (U/mg Protein)[a]
Phenylpyruvate	0.3	4.6
1	0.8	2.9
6	0.4	2.3
8	0.5	2.1
12	0.7	3.6
15	0.7	0.7
16	0.7	1.5
19	2.3	2.6

a) One unit of enzyme reduces 1 μmol 2-oxo acid per min, that corresponds to the oxidation of 2 μmol of benzylviologen cation radical ($BV^{+\cdot}$).

4-oxo acids.

The following facts are indicative for the regioselectivity of the bioreduction of 2,4-dioxocarboxylates. The consumption of hydrogen exceeded never 1 mol for one mol of substrate. The mass spectra, ^{1}H-NMR and IR spectra for **10a** and **11a** are in accordance with a 2-hydroxy-4-oxo carboxylate. 3-Oxo butyrate shows a hydrogenation rate < 0.1% of that of 2-oxo butyrate. Therefore one can assume that only the 2-oxo group of 2,4-dioxo carboxylates are reduced.

Spontaneous reaction between 2-oxo acids and reduced viologens. Concentrations of 5 mM of **1** or 2 mM of **8** show in the precence of 0.5 mM $BV^{+\cdot}$ and in the absence of the biocatalyst in oxygen free cuvettes a slow spontaneous reaction by which reduced benzylviologen seems to disappear irreversibly. 2-Hydroxy acids as products could not be observed. Such a reaction does not seem to play an essential role in the here described biological reductions. During microbial reductions the total concentration of benzylviologen was always 1 mM but the solutions were hardly colored. That means, the steady state concentration of $BV^{+\cdot}$ was rather low. At the end of a preparative reaction there was always a strong color from the $BV^{+\cdot}$ indicating the presence of much of the original 1 mM benzylviologen. No spontaneous reaction was observable with **1** or **8** and reduced carbamoylmethylviologen which has an E_o of -295 mV, about 70 mV less negative than benzylviologen. More details regarding various viologens are already published by us.[2b]

Concluding Remarks

P. vulgaris is a very versatile biocatalyst which can be easily grown also in a chemical laboratory. It possesses not only a new type of a reversible 2-hydroxy carboxylate oxido reductase but also the enzymes for a very simple regeneration of artificial cosubstrates. One liter cell culture reaching the stationary phase delivers the amount of cells by which about 30 mmol of a (R)-2-hydroxy acid can be prepared from the corresponding 2-oxo

Table 6. Conditions of HPLC analyses and retention times of various substrates and products

Compound	Column	Eluent	Flow rate	Retention time [min]
1	RP-18[a]	30% MeOH	2.0	28
1a	RP-18	30% MeOH	2.0	23.2
1a	Chiral-1	10 mM $CuSO_4$	0.5	15.7
rac-1a	Chiral-1	10 mM $CuSO_4$	0.5	12.2[c] (S); 15.7 (R)[d]
2	RP-18	30% MeOH	1.5	27.0
2a	RP-18	30% MeOH	1.5	27.9
2a	Chiral-1[b]	5 mM $CuSO_4$	0.5	12.1
rac-2a	Chiral-1	5 mM $CuSO_4$	0.5	7.5 (S); 12.0 (R)
3	RP-18	45% MeOH	2.0	42.6
3a	RP-18	45% MeOH	2.0	42.0
3a	Chiral-1	10 mM $CuSO_4$	0.3	7.8
rac-3a	Chiral-1	10 mM $CuSO_4$	0.3	6.3 (S); 7.8 (R)
4	RP-18	45% MeOH	2.0	53.0
4a	RP-18	45% MeOH	2.0	51.6
4a	Chiral-1	5 mM $CuSO_4$	0.5	10.7
rac-4a	Chiral-1	5 mM $CuSO_4$	0.5	8.7 (S); 11.1 (R)
5	RP-18	30% MeOH	2.0	17.6
5a	RP-18	30% MeOH	2.0	16.8
5a	Chiral-1	10 mM $CuSO_4$	0.3	6.45
rac-5a	Chiral-1	10 mM $CuSO_4$	0.3	5.1 (S); 6.45 (R)
6	RP-18	20% MeOH	2.0	21.6
6a	RP-18	20% MeOH	2.0	15.6
6a	Chiral-1	5 mM $CuSO_4$	0.5	6.3
rac-6a	Chiral-1	5 mM $CuSO_4$	0.5	5.5 (S); 6.5 (R)
7	RP-18	0% MeOH	1.2	12.5
7a	RP-18	0% MeOH	1.2	12.7
7a	Chiral-1	10 mM $CuSO_4$	0.5	4.2
8	RP-18	50% MeOH	1.0	13.0
9	RP-18	40% MeOH	1.0	22.6
13	RP-18	20% MeOH	2.0	14.4
13a	RP-18	10% MeOH	2.0	14.8
13a	Chiral-1	5 mM $CuSO_4$	0.3	4.6
14a	Chiral-1	5 mM $CuSO_4$	0.5	7.0
rac-14a	Chiral	5 mM $CuSO_4$	0.5	5.3 (S); 7.0 (R)
17a	RP-18	0% MeOH	1.0	14.6; 23.2

[a] Nucleosil RP-18 (Macherey & Nagel). An octadecyl residue is bound to silica gel. [b] Chiral-1 (Macherey & Nagel) provides a ligand exchange chromatography by L-hydroxy prolin Cu^{2+} ions bound to silica gel. [c] Retention volume of S-enantiomer; [d] Retention volume of R-enantiomer.

acid at the expense of formate in 300 ml dilute buffer containing 1 mM benzylviologen or 2 mM cobalt sepulchrate in about 24 h. Although the enzymes present in the resting cells are inactivated by oxygen, flushing of the reaction vessel and the solutions with nitrogen before the reaction is

started is sufficient for this scale. The smaller the scale the more elaborate precautions for the exclusion of oxygen have to be taken. That is especially true for kinetic measurements using reduced viologens as electron donors.[3a]

The range of substrates accepted by this biocatalyst at a rate useful in organic synthesis seems to be much larger than the combination of all other known 2-oxo acid reductases.[1,12] As shown earlier also 2-oxo dicarboxylates $^{-}OOC-(CH_2)_nCOCOO^{-}$ (n = 1-3) are substrates.[2a-d] We assume that also dicarboxylates with n > 3 can be reduced. The usual disadvantages of fermentations do not exist. The amount of biocatalyst is only about 0.1-1% of that which is necessary for most other bioreductions with yeasts. The K_m values are satisfyingly small. Using benzylviologen leads to an equilibrium constant $>10^7$ in favor of the 2-hydroxy acid.

The dehydrogenation of racemic mixtures of 2-hydroxy acids to a mixture of 2-oxo acid and (S)-2-hydroxy acid is possible,[2d,3b] and of preparative value. Experiments on a scale up to a few hundred mmol of this dehydrogenation will be described (publication in preparation).

Tsuboi et al.[9b] described the reduction of 3-chloro-2-oxoalkanoate esters with growing yeasts with PN values of 5-10. These substrates can not be reduced with *P. vulgaris*.

EXPERIMENTAL

General procedure. 2-Oxo acids **13-20** were obtained from commercial sources. Mp. are not corrected.

Cells of *P. vulgaris* (DSM 30 115) were grown as described[3a] and stored as wet packed cells under exclusion of oxygen at -15°C or in freeze dried form at room temperature. The lipase preparation Lipozyme^R 10 000 L from *Mucor miehei* with a standard activity of 10^4 U/g was a gift from Novo (DN-Copenhagen). Benzylviologen was bought from Aldrich and carbamoylmethylviologen was synthesized as described.[2b]

Protein was determined according to Bradford in the version of Read and Northcote.[13]

Preparation of cell free extracts and determinations of enzyme activities by initial rate studies were conducted as described.[3a] For measurements in cuvettes electrochemically reduced benzylviologen was applied as electron donor. The ratio reduced to oxidized benzylviologen was 2:1.

For measurements of optical rotations the ORD spectral photometer J5 of Jasco (J. Tokyo) was used.

Mass spectra were conducted with a Varian Mat CH 5 applying an ionization energy of 70 eV.

IR spectra of substrates and products were registered from solutions in $CHCl_3$, films or in KBr using a Perkin Elmer 157 G or 257.

^{1}H-NMR spectra were performed with a WP 200 or AM 360 spectrometer of Bruker and ^{13}C-NMR with the AM 360 spectrometer applying 90.556 MHz. If not mentioned otherwise the solvent was $CDCl_3$.

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. Depending on the hydrophobicity of the subtrates and products or derivatives 0.1% aqueous phosphoric acid containing 10-60% methanol was used as an eluent with a flow rate of 1 - 2 ml/min. For the determination of the optical purity a Chiral-1^R column (Macherey and Nagel) was used. The refractive index and uv absorption were simultaneously recorded. For hydroxy acids without additional functional groups the uv absorption at 214 nm was used. The wave lengths for monitoring other compounds were adapted according to their absorption. For analyzing unknown concentrations calibration curves were applied. Table 6 shows the chromatographical behavior of substrates and products. Usually substrates and products were purified until they were homogeneous by HPLC.

General procedure for bio reductions. Oxygen was excluded from the *P. vulgaris* cells. Before use, buffers were boiled and cooled under an atmosphere of nitrogen. Bulbs and other containers were flushed with nitrogen.

In order to judge a new substrate and to compare its reaction rate with that of the reduction of 3-phenylpyruvate the following standard procedure was applied: A total volume of 3.0 ml containing 0.3 mmol substrate, 1 mM oxidized benzylviologen, 0.1 M phosphate buffer pH 7.0 and 100 mg wet packed cells (20% dry weight) of *P.vulgaris* was shaken at 35°C in little bulbs connected to mercury filled Warburg manometers (Braun, D-3508 Melsungen) under an atmosphere of hydrogen gas. The rate of hydrogen consumption indicating the rate of product formation was registered by reading the hydrogen pressure in suitable time intervalls. If necessary besides measuring the hydrogen consumption the concentrations of substrates and products were analyzed by taking small aliquots *via* suitable syringes from the reaction mixture, heating the samples in closed Eppendorf caps for protein precipitation and injecting 5 or 10 µL on a HPLC-column.
Reductions up to 10 mmol of substrate at the expense of hydrogen gas were usually carried out by shaking the reaction mixtures in suitable vessels to which a manometer and a storage vessel for hydrogen gas was connected. As long as less than 1-2 mmol substrate was applied reductions with formate as electron donor or with hydrogen gas and formate in combination were conducted in the same manner. Substrate amounts up to 120 mmol were reduced with formate by stirring the reaction mixture in 3-necked bulbs containing a pH electrode connected with the formic acid reservoir of an automatic pH control system.

Synthesis of 2-Oxo-3-enoic acids
The E-isomers of 2-oxo-3-enoic acids were obtained. All 2-oxo acids or derivatives were checked for homogeneity by HPLC. Data are shown in Table 6. With a few modifications the procedure of Reimers[6a] was followed for the preparation of **1-6**.

2-Oxo-4-phenyl-3-butenoic acid (1). Preparation according to [6a] yielded 81%. The salt was dissolved in 800 ml water at 0°C and while stirring intensively 70 ml conc. HCl was added. The isolated acid (71% yield) was recrystallized from benzene. Mp. 69-70 °C. Anal. calcd. for $C_{10}H_8O_3$: C, 68.18; H, 4.58; Found: C, 68.20; H, 4.60.

2-Oxo-3-methyl-4-phenyl-3-butenoic acid (2). To a stirred mixture of 5.3 g 2-oxo butanoic acid (50 mmol) and 4.3 g benzaldehyde (50 mmol) at 0°C 12 ml of a solution of 4.2 g potassium hydroxide in 18 ml methanol was slowly added keeping the temperature below 5°C. After removing the ice bath the residual 6 ml KOH solution was added, leading to a temperature of 40°C which was kept for 45 min. The originally white precipitate turned yellow. The salt (82% yield) was converted to the free acid as described for 1. Mp. 98-100°C. Anal. calcd. $C_{10}H_9KO_3$: C, 55.53; H, 4.19; Found: C, 55.22; H, 4.36.
The oxo acids 3,4,5, and 6 were prepared in analogy to 1. 5 and 6 were analyzed as potassium salts. 5: Anal. calcd. for $C_8H_5KO_3S$: C, 43.62; H, 2.29; Found: C, 43.34; H, 2.33. 6: Anal. calcd. for $C_8H_5KO_4$: C, 47.05; H, 2.47; Found: C, 46.64; H, 2.56.

2-Oxo-6-phenyl-3,5-hexadienoic acid (8) was prepared on a 200 mmol scale according to 2 using cinnamic aldehyde. The yield of the salt was 89% and that of the free acid which precipitated as an oil was 93% from which only 30% crystalline material could be obtained. Mp. 102-103°C.

2-Oxo-3-methyl-6-phenyl-3,5-hexadienoic acid (9)[6c] was prepared on a 50 mmol scale according to 2 using 2-oxo butyric acid and cinnamic aldehyde. The total yield in form of the isolated salt was 52%.

2-Oxo-3-pentenoic acid (7). Under exclusion of moisture and oxygen 60.5 g (0.5 mol) 1-bromopropene in 200 ml THF was slowly added to a suspension of 13.0 g freshly activated magnesium chips with a trace of iodine in 250 ml THF. The reaction started after adding a few ml of the 1-bromopropene solution and the temperature of the mixture rose to 35°C. During further addition of 1-bromopropene the temperature was kept between 40-50°C. After 2 h reflux boiling the mixture was cooled to -75°C and 48 ml freshly destilled ethyl oxalate in 50 ml dry THF and 400 ml diethyl ether was slowly dropped into the Grignard product keeping the temperature below -70°C. The disappearance of ethyl oxalate was determined in small samples by HPLC. The temperature was raised to -55°C and 100 ml conc. sulfuric acid in 800 ml water was carefully added. After 3 h stirring the phases were separated, the aqueous phase extracted twice with 200 ml diethyl ether, the organic phase washed with 300 ml saturated sodium chloride solution and dried over sodium sulfate. After fractionated distillation (50-55°C, 8 mm Hg) 25 g 7-ethylester (58% yield based on ethyl oxalate) was obtained. An automatic pH-control system was set to pH 7.2 and a 1 M solution of KOH added to a stirred suspension of 18 g 7-ester in 10 ml water. After 15 h at room temperature the solution was washed with diethyl ether and the aqueous phase lyophilized leading to 16 g potassium salt. ^{1}H-NMR (^2H_2O): δ 2.07 (dd, J(1) = 7.0 Hz, J(2) = 1.5 Hz, 3H), 6.30 (dq, J(1) = 16.0 Hz, J(2) = 1.5 Hz, 1H), 7.20 (dq, J(1) = 16.0 Hz, J(2) = 7.0 Hz, 1H); ^{13}C-NMR (^2H_2O): 200.6, 175.4, 156.8, 130.5, 21.3.

Ethyl 2,4-dioxoacylates 10-12 were synthesized according to a general Organic Synthesis procedure of Snyder et al.[14]

Ethyl 2.4-dioxo-4-phenylbutyrate (10-ethylester). In a 0-5°C solution of 2.5 g (0.11 mol) sodium in 33 ml ethanol 13.5 ml ethyl oxalate was added and afterwards during 30 min 11.6 ml (0.1 mol) acetophenone dissolved in 10 ml ethanol. After 1 h the cooling bath was removed and the solution stirred at room temperature for 18 h. The sodium salt obtained, was dissolved in 400 ml water and washed with 200 ml diethyl ether. Under cooling the aqueous phase was acidified with 2 N HCl to pH 2 and again 3 times extracted with 150 ml ether. The residue from the dried ether solution could be crystallized from hexane/chloroform 9:1. Yield 44%, mp. 32°C, very hygroscopic.
The oxo acids 11 and 12 were prepared as 10 using 9.8 g desoxybenzoin or 15 g cyclohexanone, respectively.
17 was synthesized according to Takeda et al.[9a] Ethyl 2-chloro-2,3-epoxy-hexanoate was rearranged to ethyl 3-chloro-2-oxo-hexanoate and the latter was hydrolyzed. The ester (4.31 g = 0.022 mol) was suspended in 5 ml water and the suspension neutralized with 1 N NaOH. The hydrolysis occurred with 1 N NaOH in 22 h at room temperature using an automatic pH control system set at pH 8. About 0.04 mol NaOH were consumed. The solution was washed with 30 ml diethyl ether, acidified with sulfuric acid and continuously extracted with diethyl ether. The residue from the dried ether extract was dissolved in 10 ml water and the neutralized aqueous solution freeze dried. Yield 2.9 g (87%).

Racemates of various 2-hydroxy acids. Rac-1a. Ten mmol 1 was dissolved in 25 ml of a 0.4 M solution of $CeCl_3x7H_2O$ in methanol, under exclusion of oxygen, during 25 min. $NaBH_4$ (0.38 g ≙ 10 mmol) was added and after 10 min the reaction was stopped by adding 25 ml 0.3 N H_2SO_4. The product was extracted with diethyl ether and the crude hydroxy acid recrystallized from chloroform. Yield 74%. The retention volumes for the enantiomers of 1a and others on a Chiral-1 column are given in Table 6. From all other 2-oxo-3-enoic acids 2-6 the racemic mixture was prepared on a 1 mmol scale according to this procedure.

Examples for preparative bioreductions with formate. Variation A without using a pH-control system. Under an atmosphere of nitrogen in a two-neck round bottom flask closed with rubber septums 1.5 equivalents of sodium formate and 1 equivalent of 2-oxo acid in form of the sodium or potassium salt making the final concentration 100 mM were dissolved in 80% of the total amount of 0.1 M potassium phosphate buffer pH 7.0. Wet packed cells of *P.vulgaris* were suspended in the residual oxygen free buffer and injected into the flask through the septum. After warming to 35°C a solution of benzyl viologen was injected to reach a 1 mM concentration and the suspension stirred under a slow stream of nitrogen, passing a water trap, through an inlet and outlet syringe needle. For 10 mmol substrate 2.6 g wet packed cells of *P. vulgaris* were applied. The reaction rate slows down due to a pH drop.
Variation B. Instead of 0.1 M phosphate buffer a 0.02 M concentration is sufficient. Into the reaction mixture a pH electrode was immersed and by an automatic pH control system set to pH 7.0, 1 M formic acid was automatically added.
Variation C. The set up is as described for variation B. But after the conversion of the substrate present at the beginning at 0.1 M a second and third portion was added in form of a concentrated solution leading finally to 0.24 M product. By this procedure in 8.5 h, 0.12 mol of 1 was converted by 9.2 g wet packed cells corresponding to 1.5 g dry weight.

Reduction of 2,4-dioxo acids under *in situ* hydrolysis of the ethyl esters. Variation A was applied. The cell suspension which was added to the reaction mixture after its temperature was raised to 35°C contained per mmol of substrate 1 ml of Lypozyme^R. Per mmol substrate 2 g wet packed cells of *P. vulgaris* were used.

Isolation of the products. The reaction mixture was acidified with 4 N sulfuric acid to pH 1.8-2.0. Under diminished pressure 50-60% of the water was evaporated and the residual solution continuously extracted with diethyl ether. After drying with magnesium sulfate the ether was evaporated and the residue recrystallized.

Some properties of the hydroxy acids: 1a: Mp. 135-137°C; $[\alpha]_D$ = -90.6° (MeOH, 110 mM); ^{1}H-NMR (DMSO-d_6): δ 7.44 (d, J = 7.3 Hz, 2H), 7.34 (t, J = 7.4 Hz, 2H), 7.25 (t, J = 7.4 Hz, 1H), 6.73 (dd, J_1 = 1.1 Hz, J_2 = 15.9 Hz, 1H), 6.38 (dd, J_1 = 5.6 Hz, J_2 = 15.9 Hz, 1H), 4.71 (dd, J_1 = 1.4 Hz, J_2 = 5.6 Hz, 1H), 12.6 (br., COOH), 5.7 (br., OH). Molecular ion of MS 178 (calcd. 178). 1a prepared in ^2H_2O showed a molecular ion of 179 (calcd. after recrystallization, 179). ^{13}C-NMR: 173.9, 136.4, 130.4, 128.8(2C), 128.0, 127.8, 126.5(2C), 71.0. Anal. calcd. for $C_{10}H_{10}O_3$: C, 67.40; H, 5.66; Found: C, 67.40; H, 5.57.

2a: Mp. 125-128°C; $[\alpha]_D$ = -96.7° (MeOH, 62.5 mM); ^{1}H-NMR: δ 7.22-7.39 (m, 5H), 6.61 (s, 1H), 4.58 (s, 1H), 1.85 (d, J = 1.1 Hz, 3H). ^{13}C-NMR 173.7, 136.8, 136.1, 128.6(2C), 128.1(2C), 127.1, 126.5, 76.0, 13.8. Molecular ion of MS 192 (calcd. 192). Anal. calcd.

for $C_{11}H_{12}O_3$: C, 68.77; H, 6.25; Found: C, 68.57; H, 6.24.

3a: Mp. 133-35°C; $[\alpha]_D$ = -108,8° (MeOH); ^{1}H-NMR: δ 7.48 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 6.70 (d, J = 16 Hz, 1H), 6.40 (dd, J_1 = 16 Hz, J_2 = 5.4 Hz, 1H), 4.70 (d, J = 5.4 Hz, 1H), 5.6 (br., OH). ^{13}C-NMR (DMSO): 173.5, 135.2, 131.0, 128.8(2 peaks), 128.6, 128.0, 70.7. Molecular ion of MS 212/214 = 3:1 (calcd. 212/214). Anal. calcd. for $C_{10}H_9O_3Cl$: C, 56.49; H, 4.27; Found: C, 56.23; H, 4.28.

4a: Mp. 148-50 °C; $[\alpha]_D$ = -95.7° (MeOH, 110 mM); ^{1}H-NMR: δ 7.51 (d, J = 8.3 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 6.70 (d, J = 15.9 Hz, 1H), 6.43 (dd, J_1 = 15.9 Hz, J_2 = 5.4 Hz, 1H), 4.71 (d, J = 5.4 Hz, 1H). ^{13}C-NMR: 179.5, 135.46, 131.46(2 peaks), 128.88, 128.32, 120.53, 70.73. Molecular ion of MS 256/258 = 1:1 (calcd. 256/258). Anal. calcd. for $C_{10}H_9O_3Br$: C, 46.72; H, 3.53; Found: C, 46.48; H, 3.60.

5a: Mp. 80°C (dec); $[\alpha]_D$ = -98.0° (MeOH, 97 mM); ^{1}H-NMR (DMSO-d_6,): δ 7.41 (d, J = 5.4 Hz, 1H), 7.11 (d, J = 4.3 Hz, 1H), 7.01 (dd, J_1 = 5.1 Hz, J_2 = 4.3 Hz, 1H), 6.1 (dd, J_1 = 5.5 Hz, J_2 = 15.6 Hz, 1H), 6.77 (J_1 = 15.6 Hz, J_2 = 1 Hz, 1H), 4.65 (dd, J_1 = 5.5 Hz, J_2 = 1 Hz, 1H). ^{13}C-NMR: 173.3, 140.8, 127.5, 126.2, 124.9, 123.4, 70.2. Molecular ion of MS 184 (calcd. 184).

6a: Rather labile and air-sensitive. $[\alpha]_D$ = -28.2° (MeOH, 200 mM); ^{1}H-NMR: δ 7.38 (d, 1H), 6.60 (d, J = 15.8 Hz, 1H), 6.37 (m, 1H), 6.27 (d, 1H), 6.25 (dd, J_1 = 15.8, J_2 = 5.2 Hz, 1H), 4.73 (d, J = 5.2 Hz, 1H). ^{13}C-NMR: 173-1, 151.0, 141.0, 118.0, 110.3, 107.3, 69.5. Molecular ion of MS of methyl ester 182 (calcd. 182).

7a: Liquid after distillation contaminated with about 10% lactone formed by intramolecular addition of the carboxyl group to the C-C double bond and 5% of a dimer. $[\alpha]_D$ = -42° ($CHCl_3$, 0.14 mM) measured immediately after distillation. ^{1}H-NMR: δ 7.0 (br. 1H), 5.9 (dq 1H), 5.57 (dd 1H), 4.68 (d 1H), 1.72 (dd, J_1 = 5.4 Hz; J_2 = 1.3 Hz, 3H).

8a: Mp. 145 °C; $[\alpha]_D$ = -92° (MeOH, 50 mM); ^{1}H-NMR (DMSO-d_6): δ 7.47 (d, J = 7 Hz, 2H, o-H), 7.33 (dd, J_1 = J_2 = 7 Hz, 2H, m-H), 7.23 (tr, J = 7 Hz, 1H, p-H), 6.94 (dd, J_1 = 10.5 Hz, J_2 = 16 Hz, 1H, H_{C5}), 6.62 (d, J = 16 Hz, 1H, H_{C6}), 6.50 (ddd, J_1 = 11 Hz, J_2 = 15 Hz, J_3 = 1.2 Hz, 1H, H_{C4}), 5.98 (dd, J_1 = 15 Hz, J_2 = 5.5 Hz, 1H, H_{C3}), 4.64 (dd, J_1 = 5.5 Hz, J_2 = 1.1 Hz, 1H, H_{C2}), 5.58 (s, br., 1H, -OH). ^{13}C-NMR (90,556 MHz, DMSO-d_6): 173.7 (C-1), 136,8 (ar-C-1), 132.2, 132.0, 130.8, 128.2 and 127.5 (C-3, -4, -5, -6 and ar-C-4), 126.2 and 128.6 (ar-C-2, -6 and ar-C-3, -5), 70.6 (C-2).

9a: Mp. 141 °C; ^{1}H-NMR (DMSO-d_6): δ 12.55 (s, br., 1H, -COOH), 7.52 (d, J = 7.4 Hz, 2H, ar-H-2/6), 7.33 (dd, J_1 = 7.4 Hz, J_2 = 7.8 Hz, 2H, ar-H-3/5), 7.23 (trtr, J_1 = 1.1 Hz, J_2 = 7.5 Hz, 1H, ar-H-4), 7.12 (dd, J_1 = 11 Hz, J_2 = 15.6 Hz, 1H, -CH=C<u>H</u>-CH=), 6.61 (d, J = 15.6 Hz, 1H, Ph-C<u>H</u>=), 6.32 (d, J = 11 Hz, 1H, -C<u>H</u>=C(CH_3)-), 5.45 (s, br., 1H, -CHO<u>H</u>-), 4.52 (s, 1H, -C<u>H</u>OH-), 1.86 (s, 3H, -CH_3). ^{13}C-NMR (DMSO-d_6): 173.64 (-COOH), 136.78 and 137.17 (ar-C-3 and C-4), 124.70, 127.29, 127.39 and 132.40 (ar-C-4, C-4, C-5 and C-6), 126.30 and 128.55 (ar-C-2/6 and ar-C-3/5), 75.51 (-<u>C</u>HOH-), 12.92 (-CH_3). Molecular ion of MS 218 (calcd. 218).

10a: Mp. 142 °C; $[\alpha]_D$ = +1.84° (0.1 M, MeOH); ^{1}H-NMR (acetone-d_6): δ 8.03 (dd, J_1 = 7.5 Hz, J_2 = 1.4 Hz, 2H, o-H), 7.63 (tr, J = 7 Hz, 1H, p-H), 7.54 (dd, J_1 = J_2 = 7.5 Hz, 2H, m-H), 4.71 ('tr', J = 5.6 Hz, 1H, -C<u>H</u>OH-), 3.48 (d, J = 5.3 Hz, 2H, -CH_2-). ^{13}C-NMR (acetone-d_6): 197.9 (C-4), 175.2 (C-1), 138.1 (ar-C-1), 134.0 (ar-C-4), 129.5 and 129.0 (ar-C-2,6 and ar-C-3,5), 67.8 (C-2), 43.4 (C-3). Molecular ion of MS 194 (calcd. 194); IR (KBr) [cm^{-1}] 3460 (OH); 1730 (COOH); 1680 (CO). Anal. calcd. for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19; Found: C, 61.88; H, 5.13.

11a: Mp. 87-90°C; ^{1}H-NMR ($CDCl_3$): δ 8.09-8.11, 7.90-7.92, 7.59-7.63, 7.44-7.51 and 7.25-7.39 (m, 10H, ar-H), 5.21 (d, J = 4.1 Hz, 1H <ca. 90%>, -C<u>H</u>OH-), 5.16 (d, J = 3.6 Hz, 1H, <ca. 10%>, -C<u>H</u>OH-), 5.06 (d, J = 3.6 Hz, 1H <ca. 10%>, H_{C3}), 4.68 (d, J = 4.1 Hz, 1H <ca. 90%>, H_{C3}). ^{13}C-NMR ($CDCl_3$): 201.20 (-CO-), 172.4 and 172.1 (-COO-), 135.1 - 127.28 (C-ar), 73.07 (-CHOH- <90%>), 70.0 (-CHOH- <10%>), 68.3 (C-3 <10%>), 56.53 (C-3 <90%>). IR (KBr) [cm^{-1}] 1750 (COOH); 1690 (CO).

12a: Material partily seems to cyclize during recrystallization to a product which could be a 5-ring lactone. The crude product showed [α] = -58°(0.22 M CH_2Cl_2).

14a: Mp. 57-59°C; $[\alpha]_D$ = -4.3° ($CHCl_3$, 12.8 mM); ^{1}H-NMR ($CDCl_3$) : δ 7.15 (broad, 1H; OH); 4.27 (dd, J_1 = 4.3 Hz, J_2 = 7.5Hz, 1H; -C<u>H</u>OH-); 1.6-1.72 and 1.75-1.9 (per m 1H; -C<u>H</u>$_2$-CHOH-); 1.45 (m, 2H; H_{C4}); 1.29 (m, 6H; H_{C5} to H_{C7}); 0.88 (tr, J= 7Hz, 3H; CH_3). ^{13}C-NMR $CDCl_3$: δ 179.8 (C-1); 70.4 (C-2); 34.1, 31.6, 28.9, 24.7, 22.6 and 14.0 (C-3 to C-8). Anal. calcd. for $C_8H_{16}O_3$: C, 59.97; H, 10.07; Found: C, 60.04; H, 9.71.

17a: 1:1-Mixture of (R,R) - and (R,S)-2,3-dihydroxyhexanoic acid. Oily residue after evaporation of diethyl ether used for extraction. ^{1}H-NMR (acetone-^{2}H): δ 9.42 (s, broad, 1H, - COOH); 4.55 and 3.71 (d, J_1 = 7 Hz, J_2 = 13 Hz, 1H, H_{C2}); 4.37 and 4.14 (per m 1H, H_{C3}); 1.3 - 1.8 (m, 2 x 4H, $-CH_2-CH_2-$); 0.95 and 0.92 (tr, J = 7.4 Hz, 3H, $-CH_3$). ^{13}C-NMR δ 172.6 and 172.1 (C-1); 79.8, 79.7, 78.0 and 77.7 (C-2 and C-3); 36.5 and 34.3 (C-4); 20.3 and 19.7 (C-5); 14.3 (C-6).

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