α Hydroxylation of Carboxylic Acids with Molecular Oxygen Catalyzed by the α Oxidase of Peas (*Pisum sativum*): A Novel Biocatalytic Synthesis of Enantiomerically Pure (*R*)-2-Hydroxy Acids

Waldemar Adam,[†] Wilhelm Boland,[§] Jenny Hartmann-Schreier,[‡] Hans-Ulrich Humpf,[‡] Michael Lazarus,*[,‡] Alexander Saffert,[‡] Chantu R. Saha-Möller,[†] and Peter Schreier[‡]

Contribution from the Institutes of Organic Chemistry and of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany, and the Max-Planck Institute of Chemical Ecology, Tatzendpromenade 1a, D-07745 Jena, Germany

Received April 14, 1998

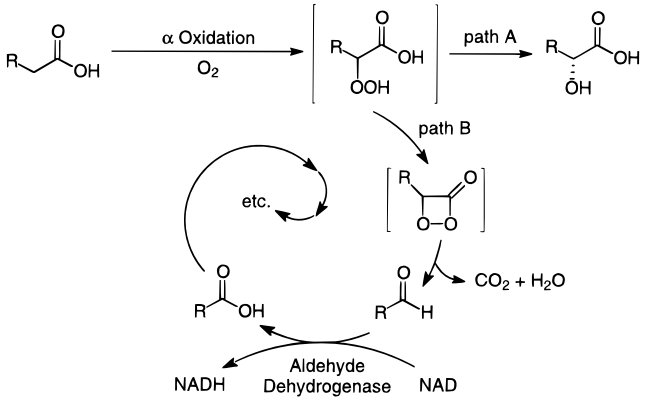
Abstract: The substrate selectivities of the α oxidation of saturated, unsaturated, and heteroatom-containing (oxygen, sulfur) carboxylic acids **1** by the enzyme extract of peas (*Pisum sativum*) indicate that this biotransformation proceeds highly enantioselectively. For the first time, the synthesis of optically pure 2-hydroxy acids **2** has been achieved on the semipreparative scale (1 mmol) by α hydroxylation of long-chain carboxylic acids with molecular oxygen, catalyzed by the α oxidase of peas. For derivatives with sulfur atom in the chain, no sulfoxidation is observed. The functionalities (carbon double and triple bonds, oxygen, and sulfur atoms) must be at least three carbon atoms away from the carboxylic acid group to achieve efficient asymmetric hydroxylation. The absolute configuration of the 2-hydroxy acids **2** was assigned by comparison of the gas-chromatographic data with that of authentic reference compounds and by application of the exciton-coupled-circular-dichroism (ECCD) method. This unprecedented asymmetric biocatalytic methodology should be valuable for the preparation of enantiomerically pure (*R*)-2-hydroxy acids.

Introduction

The α oxidation of fatty acids is known for higher plants such as pea leaves (*Pisum sativum*),[1] germinating peanuts (*Arachis hypogaea*),[2] cucumbers (*Cucumis sativus*),[3] and potatoes (*Solanum tuberosum*),[4] as well as for simple organisms such as marine green algae (*Ulva pertusa*).[5] In higher plants, 2-hydroxy fatty acids are formed in the oxidative lipid metabolism by α oxidation of the corresponding acids.[2,6]

The mechanism of this biochemical reaction was worked out by Shine and Stumpf [2] in the seventies (Scheme 1). They have postulated that the flavoprotein-catalyzed oxidation of the fatty acid leads to an intermediary α-hydroperoxy acid, which preferentially decarboxylates to the corresponding aldehyde (Scheme 1, path B) in competition with reduction to the 2-hydroxy acid (Scheme 1, path A). Presumably, the 2-hydroperoxy acid is first converted to an intermediary α-peroxy lactone, which decomposes very fast to CO_2 and the aldehyde. Evidence for the α-peroxy lactone has been inferred by the fact that chlorophyll could be chemically excited when present in this α-oxidation process.[6,7] While the 2-hydroxy acid accumulates, the aldehyde is oxidized by an NAD^+-dependent aldehyde dehydrogenase to the next lower homologous fatty acid, which in turn functions as a substrate for the α oxidation.[2,6] In the case of hexadecanoic (palmitic) acid, the (*R*)-2-hydroxyhexadecanoic acid was formed enantioselectively.[8] Unfortunately, the membrane-bound α oxidase has not yet been isolated in pure form and, therefore, its structure elucidation is still pending.

Scheme 1. α Oxidation of Fatty Acids in Higher Plants



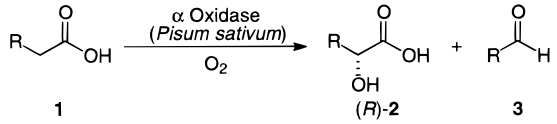
Chiral α-hydroxy acids are important building blocks for the synthesis of optically active glycols,[9a] halo esters,[9b] and epoxides.[9c] For this purpose, several chemical[10] and enzymatic[11]

[†] Institute of Organic Chemistry, University of Würzburg.
[§] Max-Planck Institute of Chemical Ecology.
[‡] Institute of Pharmacy and Food Chemistry, University of Würzburg.

(1) Hitchcock, C.; James, A. T. *Biochim. Biophys. Acta* **1966**, *116*, 413−424.
(2) Shine, W. E.; Stumpf, P. K. *Arch. Biochem. Biophys.* **1974**, *162*, 147−157.
(3) Galliard, T.; Matthew, J. A. *Biochim. Biophys. Acta* **1976**, *424*, 26−35.
(4) Laties, G. G.; Hoelle, C. *Phytochemistry* **1967**, *6*, 49−57.
(5) Kajiwara, T.; Yoshikawa, H.; Saruwatari, T.; Hatanaka, A.; Kawai, T.; Ishihara, M.; Tsuneya, T. *Phytochemistry* **1988**, *27*, 1643−1645.
(6) (a) Salim-Hanna, M.; Campa, A.; Cilento, G. *Photochem. Photobiol.* **1987**, *45*, 849−854. (b) Campa, A.; Salim-Hanna, M.; Cilento, G. *Photochem. Photobiol.* **1989**, *49*, 349−354.
(7) Schuster, G. B. *Acc. Chem. Res.* **1979**, *12*, 366−373.
(8) (a) Morris, L. J.; Hitchcock, C. *Eur. J. Biochem.* **1968**, *4*, 146−148. (b) Hitchcock, C.; Rose, A. *Biochem. J.* **1971**, *125*, 1155−1156. (c) Markovetz, A. J.; Stumpf, P. K.; Hammarström, S. *Lipids* **1972**, *7*, 159−164.

10.1021/ja981252r CCC: $15.00
Published on Web 10/14/1998

Scheme 2. α Hydroxylation of Carboxylic Acids with Molecular Oxygen Catalyzed by the α Oxidase from Peas (*Pisum Sativum*)



1 (*R*)-**2** **3**

a: R = $CH_3(CH_2)_{11}$; **b**: R = CH_2=$CH(CH_2)_7$;
c: R = (*Z*)-$CH_3(CH_2)_4CH$=$CHCH_2$; **d**: R = (*E*)-$CH_3(CH_2)_4CH$=$CHCH_2$;
e: R = (*Z*)-$CH_3(CH_2)_{10}CH$=$CHCH_2$; **f**: R = (*E*)-$CH_3(CH_2)_{10}CH$=$CHCH_2$;
g: R = (*Z*)-$CH_3(CH_2)_9CH$=$CH(CH_2)_2$; **h**: R = (*E*)-$CH_3(CH_2)_9CH$=$CH(CH_2)_2$;
i: R = (*Z*)-$CH_3(CH_2)_7CH$=$CH(CH_2)_6$; **j**: R = (*E*)-$CH_3(CH_2)_7CH$=$CH(CH_2)_6$;
k: R = $CH_3(CH_2)_4C{\equiv}CCH_2$;
l: R = $CH_3(CH_2)_{10}C{\equiv}CCH_2$; **m**: R = $CH_3(CH_2)_9C{\equiv}C(CH_2)_2$;
n: R = $CH_3(CH_2)_7OCH_2$; **o**: R = $CH_3(CH_2)_9O(CH_2)_2$;
p: R = $HOOC(CH_2)_7$; **q**: R = $CH_3OOC(CH_2)_7$;
r: R = $CH_3CH_2S(CH_2)_9$; **s**: R = $CH_3(CH_2)_6S(CH_2)_6$

methods have been reported previously on the synthesis of optically active α-hydroxy acids, whereas the biocatalytic techniques utilize oxyfunctionalized substrates. Prior to our efforts, nothing was known on the synthesis of optically active 2-hydroxy acids by the enzymatic CH-insertion from readily available carboxylic acids.

To assess the scope of the enzymatic α oxidation for the preparation of enantiomerically pure 2-hydroxy acids, we have studied in detail the α-oxidation system of peas (Scheme 2). In this context, we have recently reported our results on the substrate selectivities for the enantioselective α hydroxylation of saturated carboxylic acids with molecular oxygen catalyzed by the α-oxidation-enzyme system of young pea leaves.[12] Because of the low enzyme activity in the pea leaves, the α oxidation with the crude homogenate was only possible on the analytical scale and the aldehyde **3** was produced preferentially. Herein, we present the first enantioselective α hydroxylation of functionalized carboxylic acids with molecular oxygen on the semipreparative scale (1 mmol), catalyzed by the α oxidases of germinating peas. This novel biocatalytic method affords exclusively enantiomerically pure (*R*)-2-hydroxy acids from carboxylic acids, in particular long-chain fatty acids, when the enzymatic autoxidation is conducted in the presence of tin(II) chloride as reducing agent.

Results and Discussion

The substrate selectivity studies of the biocatalytic α oxidation of functionalized carboxylic acids were carried out under optimized conditions[12] with the crude homogenate of young pea leaves in 0.2 M phosphate buffer (pH 6.0) with Triton X-100 as emulsifier (Method A). The results are given in Table 1, wherein the conversion of the carboxylic acids **1a-s** to the 2-hydroxy acids **2a-s** and the next lower aldehyde **3**, as well as the enantiomeric excess of the 2-hydroxy carboxylic acids **2a-s**, was assessed by gas chromatography. In Table 1, the optimized reaction times are given, beyond which no further conversion of the carboxylic acids **1a-s** was observed.

Table 1. α Oxidation of Carboxylic Acids **1** with the Crude Homogenate of Young Pea Leaves (Method A) and the Crude Extract of Germinating Peas (Method B)[a]

entry	substrate (mmol)	method	time (h)	convn (%)	product ratio (%)[b] **2**:**3**	ee (%)[c] (*R*)-**2**
1	**1a** (0.02)	A	6	41	31:69	>99
2	**1a**[d] (0.1)	B	47	100	99:1	>99
3	**1a**[d] (0.5)	B	71	100	80:20[e]	>99
4	**1b** (0.04)	A	22	57	45:55	>99
5	**1d** (0.05)	A	22	40	*f*	>99
6	**1g** (0.07)	A	23	10	20:80	>99
7	**1h** (0.07)	A	23	67	17:83	>99
8	**1i** (0.03)	A	22	86	24:76	>99
9	**1j** (0.03)	A	23	22	16:84	>99
10	**1m** (0.05)	A	22	<10	65:35	>99
11	**1o** (0.06)	A	23	87	49:51	>99
12	**1q** (0.03)	A	23	17	70:30	>99[g]
13	**1r** (0.2)	B	23	100	99:1	>99
14	**1r** (0.2)	B	23	100	74:26	>99
15	**1s** (0.02)	B	24	100	99:1	>99

[a] Method A: 0.2 M phosphate buffer (pH 6.0), 0.1% Triton X-100. Method B: 0.1 M Tris−HCl (pH 6.0). [b] The product distribution, normalized to 100% conversion, was determined by GC analysis; error limits ±2%. [c] The ee values were determined by GC analysis after esterification with the Mosher reagent; error limits ±2%. [d] $SnCl_2$ (entry 2: 1.0 equiv; entry 3: 0.5 equiv) was added to the solution. [e] At long reaction times, the aldehyde **3** is transformed by oxido-reductases in the crude enzyme preparation to the corresponding alcohol **4** and carboxylic acid **5** (cf. Figure 1). [f] Only the 2-hydroxy acid was detected as product of the α oxidation. [g] The ee values were determined by GC analysis after esterification with (−)-menthyl chloroformate; error limits ±2%.

The α oxidation of the unsaturated undec-10-enoic acid **1b** and the *cis*- and *trans*-oleic acids (**1i** and **1j**) by the crude homogenate yielded exclusively the corresponding (*R*)-2-hydroxy acid **2** and the next lower aldehyde **3**, with higher preference for the latter (Table 1, entries 4, 8, and 9). It was, therefore, of interest to investigate the influence of the carbon double bond in close proximity to the carboxylic acid functionality. As shown in Table 1 (entries 6 and 7), the diastereomeric pair cis- and *trans*-hexadec-5-enoic acids (**1g** and **1h**) is oxidized to the 2-hydroxy acids **2g** and **2h** and the corresponding aldehydes **3** in ratios of 20:80 and 17:83 at 10% and 67% conversion, but the *cis*- and *trans*-hexadec-4-enoic acid pair **1e**,**f** (not shown in Table 1) was not accepted by the pea-leaf oxidase. Moreover, while the *trans*-dec-4-enoic acid (**1d**) is a substrate (Table 1, entry 5) for this enzyme to afford the *trans*-2-hydroxydec-4-enoic acid (**2d**), its geometrical isomer *cis*-dec-4-enoic acid (**1c**) is not converted (not shown in Table 1).

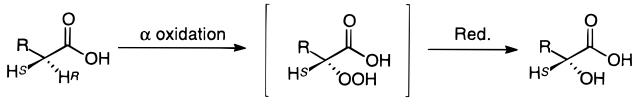
(9) (a) Prelog, V.; Wilhelm, M.; Bright, D. B. *Helv. Chim. Acta* **1954**, *37*, 221−224. (b) Lee, J. B.; Downie, I. M. *Tetrahedron* **1967**, *23*, 359−363. (c) Mori, K.; Takigawa, T.; Matsuo, T. *Tetrahedron* **1979**, *35*, 933−940.

(10) (a) Evans, D. A.; Morrissey, M. M.; Dorow, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4346−4348. (b) Corey, E. J.; Link, J. O.; Shao, Y. *Tetrahedron Lett.* **1992**, *33*, 3435−3438.

(11) (a) Wong, C.-H.; Matos, J. R. *J. Org. Chem.* **1985**, *50*, 1992−1994. (b) Nakamura, K.; Inoue, K.; Ushio, K.; Oka, S.; Ohno, A. *J. Org. Chem.* **1988**, *53*, 2589−2593. (c) Effenberger, F. *Angew. Chem.* **1994**, *106*, 1609−1619. (d) Sugai T.; Ohta, H. *Tetrahedron Lett.* **1991**, *32*, 7063−7064. (e) Adam, W.; Lazarus, M.; Boss, B.; Saha-Möller, C. R.; Humpf, H.-U.; Schreier, P. *J. Org. Chem.* **1997**, *62*, 7841−7843. (f) Adam, W.; Fell, R. T.; Hoch, U.; Saha-Möller, C. R.; Schreier, P. *Tetrahedron: Asymmetry* **1995**, *6*, 1047−1050.

(12) Adam, W.; Lazarus, M.; Saha-Möller, C. R.; Schreier, P. *Tetrahedron: Asymmetry* **1997**, *7*, 2287−2292.

Scheme 3. Selective α Oxidation of the Prochiral α Methylenic Group



Similar results were obtained for the α oxidation of alkynoic acids. While the dec-4-ynoic acid (**1k**) and hexadec-4-ynoic acid (**1l**) were not accepted as substrates by the α-oxidation enzyme (not shown in Table 1), the hexadec-5-ynoic acid (**1m**) was converted to the 2-hydroxy acid **2m** and the aldehyde **3m** (Table 1, entry 10). In the case of the alkyloxy derivatives of the propionic and butyric acid, the 3-octyloxypropanoic acid (**1n**) was not oxidized (not shown in Table 1), but the 4-decyloxybutanoic acid (**1o**) was transformed to the enantiomerically pure (*R*)-2-hydroxy acid **2o** and the aldehyde **3o** in a ratio of 49:51 at 87% conversion (Table 1, entry 11). Although sebacic acid (**1p**) resists α oxidation by this enzyme system, its methyl ester **1q** is α-hydroxylated (Table 1, entry 12), albeit at a significantly diminished reaction rate. Thus, these results show that fatty acids with less than three carbon atoms between the carboxylic acid group and functionalities such as a triple or double bond and also a heteroatom are generally not accepted by this enzymatic α-oxidation system. Furthermore, more hydrophilic carboxylic acids with short alkyl chains[12] or the dicarboxylic acids **1p** and **1q** represent very poor substrates.

A more detailed study was conducted with myristic acid (**1a**), one of the best substrates for this enzyme system. Thus, its α-oxidase-catalyzed hydroxylation with crude homogenate at pH 6 led to the enantiomerically pure (*R*)-2-hydroxymyristic acid (**2a**) and the aldehyde **3a** in a ratio of 31:69 (Table 1, entry 1) at 41% conversion.[12] Since the α-oxidase activity of this crude homogenate (grown for 14 days, Method A) was quite low and the optically active (*R*)-2-hydroxy acids **2** only accessible on the analytical scale, the crude enzyme extract of germinating peas (germinated for 3 days, Method B) was employed.

The results in Table 1 (entries 2 and 3) show that this enzyme preparation exhibits a significantly higher α-oxidase activity toward fatty acid **1a**. Nevertheless, to achieve its complete conversion to the 2-hydroxy acid **2a**, the reaction conditions had to be optimized for this model substrate (cf. Supporting Information, Table 2). Since the α oxidation is known[6,13] to proceed through a 2-hydroperoxy acid intermediate (Scheme 1), reaction conditions which favor the reduction over decarboxylation were required to increase the yield of the 2-hydroxy acid. Several reducing additives were tried out for this purpose. While cysteine or the glutathione peroxidase (GPX)/glutathione (GSH) system did not help, fortunately, the α oxidation yielded exclusively the 2-hydroxy acid **2a** in the presence of tin(II) chloride, which was successfully utilized in the in-situ reduction of fatty acid hydroperoxides formed by lipoxygenase-catalyzed autoxidation.[14] Under optimized reaction conditions, the myristic acid (**1a**) was completely converted to the enantiomerically pure (*R*)-2-hydroxy acid **2a** (Table 1, entry 2). These results demonstrate that the α oxidase of pea autoxidizes selectively the C−H^R bond of the prochiral α methylenic group in carboxylic acids (Scheme 3). Thus, the observed enantioselectivity of the α oxidation does not originate from the asymmetric reduction of the intermediary hydroperoxy acid through peroxidase activity in the crude homogenate of germinating peas.

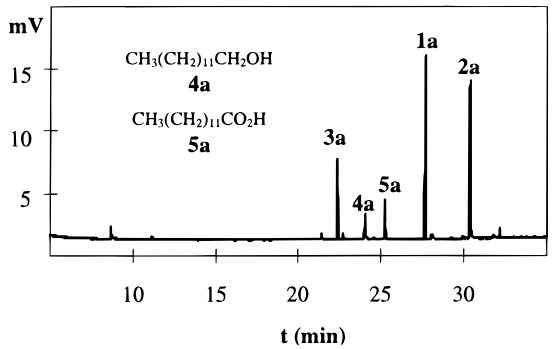


Figure 1. Product distribution, determined by GC analysis, for the α oxidation of myristic acid (**1a**) with the crude extract of germinating peas.

Unquestionably, a significant step forward was accomplished in developing this asymmetric α oxidation of long-chain carboxylic acids into a useful preparative synthesis of optically active 2-hydroxy acids. Indeed, to illustrate the synthetic value of this novel biotechnological methodology, this α oxidation was conducted on 0.5 mmol (100 mg) of the fatty acid **1a** (Table 1, entry 3; *cf.* Supporting Information, Table 2). After about 3 days of reaction time, the resulting 2-hydroxymyristic acid (**2a**) was obtained enantiomerically pure and isolated in 46% yield. The homologous aldehyde **3a** was formed as the minor product, which was transformed at long reaction times (3 days) by oxido reductases in the crude homogenate to the corresponding alcohol **4a** and carboxylic acid **5a**, as confirmed by GC analysis (Figure 1).

Also the 12-thiamyristic (**1r**) and 9-thiapalmitic (**1s**) acids were treated with the crude extract of germinating peas (Table 1, entries 13−15) to examine whether such thioether functionality survives in view of its ease of sulfoxidation.[15] Amazingly, on the analytical scale, these thia-substituted acids were exclusively and completely converted to the enantiomerically pure (*R*)-2-hydroxy acids **2r** and **2s** (entries 13 and 15). In fact, this α oxidation of the 12-thiamyristic acid (**1r**) on the semipreparative scale (ca. 50 mg, 0.2 mmol) yielded preferentially the enantiomerically pure 2-hydroxy acid **2r** at 100% conversion (entry 14); not even traces of sulfoxidation were noted.

The enantiomeric excess of the 2-hydroxy acids **2** was determined by gas chromatography on chiral columns after esterification of their methyl esters with either (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (Mosher reagent)[16] or (−)-menthyl chloroformate.[17] The elution order of the diastereomeric derivatives of the 2-hydroxy acids **2a**,**d**,**i**,**j**,**o**,**q** was ascertained on an achiral stationary phase by comparing the gas-chromatographic data with that of the authentic reference compounds.[11e,18,19] The configurations of the 2-hydroxy acids **2b**,**g**,**h**,**m** were assigned accordingly. For the determination of the configuration of the 2-hydroxy-12-thiamyristic acid (**2r**), we have employed the exciton-coupled-circular-dichroism (EC-

(13) Kajiwara, T.; Matsui, K.; Akakabe, Y. In *Biotechnology for Improved Foods and Flavours*; Takeoaka, G. R., Teranishi, R., Williams, P. J., Kobayashi, A., Eds.; American Chemical Society: Washington, 1996; pp 146−166.

(14) Koljak, R.; Boutaud, O.; Shieh, B.-H.; Samel, N.; Brash, A. R. *Science* **1997**, *277*, 1994−1996.

(15) Kagan, H. B. In *Catalytic Asymmetric Synthesis*; Ojima, I., Ed.; VCH Publishers: New York, 1993; pp 203−226.

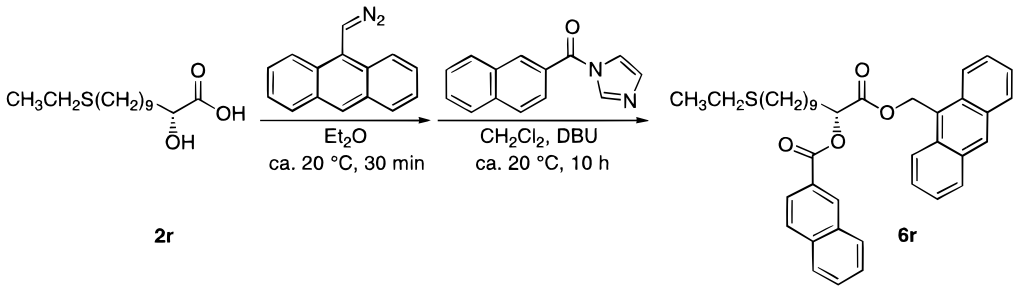
(16) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543−2549.

(17) Rees, L.; Suckling, C. J.; Wood, H. C. S. *J. Chem. Soc., Chem. Commun.* **1987**, 470−472.

(18) Adam, W.; Lazarus, M.; Schmerder, A.; Humpf, H.-U.; Saha-Möller, C. R.; Schreier, P. *Eur. J. Org. Chem.* **1998**, 2013−2018.

(19) Hamberg, M. *Anal. Biochem.* **1971**, *43*, 515−526.

Scheme 4. Functionalization of the 2-Hydroxy Acid (*R*)-**2r** to the Corresponding Bichromophoric Derivative (*R*)-**6r** for Configurational Analysis



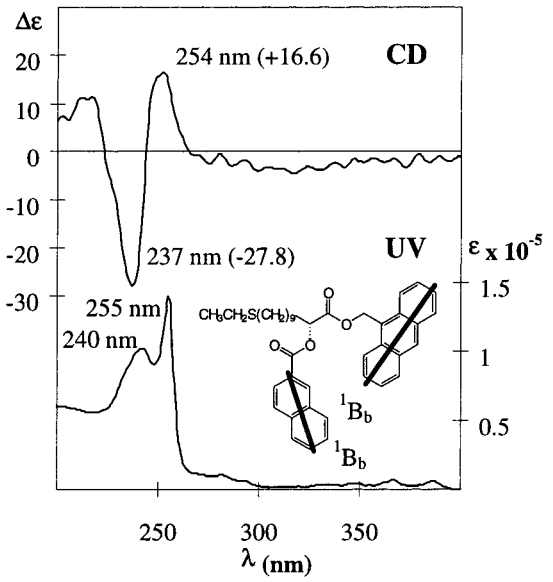


Figure 2. CD and UV spectra of the bichromophoric diester (*R*)-**6r** in acetonitrile (1-cm cell); the bold lines represent the direction of the transition dipoles.

CD)[20] method established for acyclic α-hydroxy acids.[21] After derivatization (Scheme 4) of the enantiomerically pure 2-hydroxy acid **2r** (cf. Table 1, entry 14) to the corresponding 9′-methylanthryl (*R*)-2-(2″-naphthoyloxy)-12-thiatetradecanoic acid (**6r**), the CD and UV[22] spectra of (*R*)-**6r** were recorded (Figure 2). A positive split CD curve was obtained, with extrema at 254 ($\Delta\epsilon = +16.6$) and 237 nm ($\Delta\epsilon = -27.8$) and an amplitude *A* of +44.4. This positive CD couplet suggests the *R* configuration.[21]

In summary, our results show that the α-oxidation enzyme system of peas (*Pisum sativum*) asymmetrically α-hydroxylates a broad variety of fatty acids on the semipreparative scale. As substrates serve saturated fatty acids with 7 to 16 carbon atoms[12] and the unsaturated fatty acids **1b**, **1d**, **1g**-**j**, and **1m**, as well as the heteroatom-containing (oxygen, sulfur) carboxylic acids **1o**, **1q**, **1r** and **1s**. However, when the triple and double bond or heteroatom are proximate to the carboxylic acid functionality (less than three carbon atoms), the α hydroxylation of such carboxylic acids does not take place. Also substrates without a substantially hydrophobic moiety, as is the case for the short-chain alkanoic acids[12] and the dicarboxylic acids **1p** and **1q**, are not converted by the enzyme. Despite these limitations, the α oxidation of long-chain carboxylic acids provides a convenient synthesis of enantiomerically pure (*R*)-2-hydroxy acids. Under optimized reaction conditions (pH 6 and $SnCl_2$ as reductant), the α oxidation by the crude extract of germinating peas produces *R*-enantioselectively the 2-hydroxy acid **2**, in which the reduction of the intermediary 2-hydroperoxy acid is favored over its decomposition to CO_2 and to the aldehyde **3**. In this context, the recently reported[23] purification and structure elucidation of a cucumber enzyme with high α-oxidation activity should be instrumental in understanding the mechanism of α-oxidase action. The biotechnological production of such enzymes in large amounts should foster the practical utility of this unprecedented biocatalytic method.

Experimental Section

Procedure for the Enzymatic α Oxidation of Carboxylic Acids 1 by Molecular Oxygen. Method A: To facilitate the dissolution of the solid carboxylic acids in the aqueous medium, the particular substrate was first taken up in ethyl ether (1 mL), the solvent was evaporated under reduced pressure (20 °C, 17 Torr), 2 mL of phosphate buffer (pH 6), which contained 0.1% Triton X-100, was added to the residue, and the mixture was sonicated for 1 min. The liquid carboxylic acids were directly dissolved in the phosphate buffer (pH 6) and 0.1% Triton X-100. The crude homogenate was prepared by homogenizing 10−15 g of pea leaves (var. *sativum*, grown for 14 days) with 150 mL of 0.2 M phosphate buffer and 0.1% Triton X-100 in a blender for 45 s. The aqueous carboxylic acid solution and the crude enzyme homogenate were stirred together for several hours at 4 °C, while a slow stream of oxygen gas was passed continually through the reaction mixture. Subsequently, the insoluble materials were removed by filtration, and the filtrate was acidified with 6 N hydrochloric acid (pH 3) and extracted with ethyl ether (3 × 75 mL). The combined organic phases were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure (20 °C, 17 Torr). The free acids **1** and **2** were converted to their methyl esters with diazomethane. After determination of the amount of conversion and the product distribution by GC and GC-MS analysis, the crude reaction mixture was submitted to column chromatography (silica gel, 0.032−0.062 mesh, 7:3 mixture of petroleum ether/ethyl ether) and the methyl 2-hydroxy ester was isolated in pure form. The enantiomeric excess (ee) of the methyl 2-hydroxy esters was determined by quantitative gas chromatography after derivatization with (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (Mosher reagent)[16] or (−)-menthyl chloroformate.[17]

Method B: The carboxylic acid solution was prepared as described in Method A, but instead of the phosphate buffer, the acids were dissolved in 2 M tris(hydroxymethyl)aminoethane (Tris−HCl, pH 10.3). The crude extract was prepared by homogenizing 20 g of germinating peas (germinated for 3 days) with 100 mL of 0.1 M Tris−HCl (pH 7.5) in a mortar. Subsequently, the crude homogenate was centrifuged at 9000 g for 7 min. After the aqueous carboxylic acid solution was added to the supernatant (15 mg protein/mL), the resulting

(20) Nakanishi, K.; Berova, N. In *Circular Dichroism. Principles and Applications*; Nakanishi, K., Berova, N., Woody, R. W., Eds; VCH Publishers: New York, 1994; pp 361−398.

(21) (a) Gimple, O.; Schreier, P.; Humpf, H.-U. *Tetrahedron: Asymmetry* **1997**, *8*, 11−14. (b) Hör, K.; Gimple, O.; Schreier, P.; Humpf, H.-U. *J. Org. Chem.* **1998**, *63*, 322−325.

(22) $\epsilon_{254} = 140\,000\ M^{-1}\ cm^{-1}$ for the methylanthryl chromophore (1-cm cell, CH_3CN).

(23) Andersen Borge, G. I.; Slinde, E.; Nilsson, A. *Biochim. Biophys. Acta* **1997**, *1344*, 47−58.

reaction mixture was adjusted to pH 6, blended with 3 mL of 1 M phosphate buffer (pH 6), and stirred at 4 °C under 0.4 MPa O_2 in an Amicon Ultrafiltration Cell (Model 8200), in which the filtration membrane was substituted by a Teflon sheet. Aliquots of 5 mL were removed and worked up to monitor the progress of substrate conversion. For this purpose, the solution was acidified with 6 N hydrochloric acid (pH 3) and extracted with ethyl ether (3 × 5 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure (20 °C, 17 Torr). The acids **1** and **2** were converted to their methyl esters by treatment with diazomethane and submitted to GC and GC-MS analysis. The enantiomeric excess (ee) was determined as described above.

Scale-up of the α Oxidation of Carboxylic Acid 1a in the Presence of Molecular Oxygen. The aqueous carboxylic acid solution was prepared as described under Method B, and the crude extract was obtained by homogenizing 100 g of germinating peas (germinated for 3 days) with 400 mL of 0.1 M Tris−HCl (pH 7.5) in a mortar. Subsequently, the crude homogenate was centrifuged at 9000 g for 7 min. After the aqueous carboxylic acid solution and $SnCl_2$ (0.5 equiv) were added to the supernatant, the pH of the resulting reaction mixture was adjusted to 6, blended with 12 mL of 1 M phosphate buffer (pH 6), and stirred at room temperature (ca. 20 °C) under 0.4 MPa O_2 in an Amicon Ultrafiltration Cell (Model 8400), in which the membrane filter was replaced by a Teflon sheet. Aliquots (5 mL) were removed and worked up as described under Method B to monitor the progress of substrate conversion. After 71 h of reaction time the conversion was complete, the mixture was extracted with ethyl ether (3 × 75 mL), and the combined organic layers were dried over Na_2SO_4. The solvent was removed (20 °C, 17 Torr), the products were submitted to column chromatography (silica gel, 0.032−0.062 mesh, 80:20:1 mixture of petroleum ether/ethyl acetate/acetic acid), and the 2-hydroxymyristic acid (**1a**) was isolated in pure form in 46% yield. A sample (1 mg) of the purified 2-hydroxy acid **2a** was methylated with diazomethane for analysis by capillary gas chromatography−mass spectrometry. (*R*)-**2a**: $[\alpha]^{20}_D$ −2.7 (c 1.5, $CHCl_3$) (lit.[24] $[\alpha]^{25}_D = -3.1$ (c 1.0, $CHCl_3$)). Methyl ester of **2a**: HRGC-MS, *m*/*z* (%) 199 (26), 127 (11), 125 (17), 111 (39), 103 (15), 97 (74), 90 (41), 83 (88), 81 (23), 71 (32), 69 (100), 67 (24), 57 (76), 55 (82), 43 (62), 41 (56).

Acknowledgment. This work was supported by the Bayerische Forschungsstiftung (Bayerischer Forschungsverbund Katalyse-FORKAT), the Deutsche Forschungsgemeinschaft (SFB 347, Selektive Reaktionen Metall-aktivierter Moleküle), and the Fonds der Chemischen Industrie. J.H.-Sch. thanks the University of Würzburg for a fellowship (HSP II).

Supporting Information Available: Experimental Section and Table 2 (8 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA981252R

(24) Horn, D. H. S.; Hougen, F. W.; von Rudloff, E.; Sutton, D. A. *J. Chem. Soc.* **1954**, 177−180.