Fungal Aerobic Reductive Dechlorination of Ethyl 2-Chloroacetoacetate by *Saccharomyces cerevisiae*: Mechanism of a Novel Type of Microbial Dehalogenation

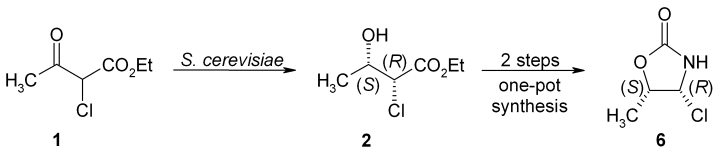
Gerhard Jörg[a] and Martin Bertau*[a]

Saccharomyces cerevisiae *reduces the β-keto ester ethyl 2-chloroacetoacetate to the respective chiral* cis- *and* trans-*β-hydroxy esters. In the course of chiral reduction, competing dehalogenation of the xenobiotic substrate to ethyl acetoacetate occurs, in a reaction mediated by cytosolic glutathione (GSH). Mechanistically, the dechlorination is a novel type of glutathione-dependent dehalogenation catalysed by an as yet unidentified glutathione-dependent dehalogenase. The first step consists of a nucleophilic replacement of the chloride substituent by glutathione. In the subsequent enzyme-catalysed step, a second glutathione molecule liberates the dehalogenation product by thiolytic attack at the thioether bridge, resulting in a net transfer of two electrons to the substrate and in the formation of glutathione disulfide (GSSG). Being effective under aerobic conditions and catalysed by a fungus, this reductive dechlorination of an aliphatic substrate is an outstanding example of a novel, glutathione-mediated microbial dehalogenation.*

Introduction

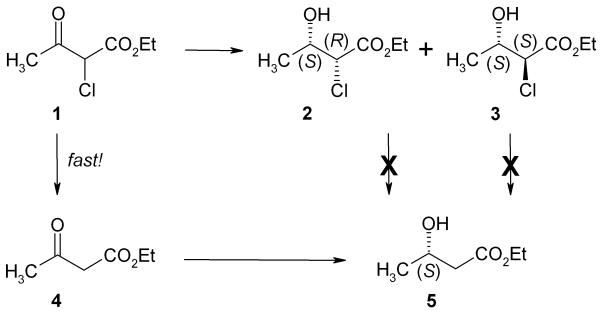
Unconventionally substituted 2-oxazolidinones of type **6** are essential substructures in novel antibiotics against multidrug-resistant Gram-positive bacteria,[1–3] in novel macrolide antibiotics,[4, 5] in renin inhibitors[6, 7] and in monoamine oxygenase inhibitors.[8, 9] However, chiral 4,5-disubstituted 2-oxazolidinones are difficult to prepare in high stereoisomeric excess,[10, 11] as a result of which there is a growing demand for new synthetic technologies.[8, 12]

We investigated the microbial reduction of ethyl 2-chloroacetoacetate with the purpose of producing the 4,5-bisfunctionalised chiral 2-oxazolidinone **6** from **1** (Scheme 1) according to a novel protocol developed in our group.[13]



Scheme 1. *Synthesis of the chiral, pharmacologically essential 2-oxazolidinone subunit* ***6*** *by stereoselective microbial reduction of the β-keto ester* ***1****.*

The required high stereopurity of β-hydroxy ester **2** can only be achieved by biocatalytic methods, with whole-cell biotransformation with *Saccharomyces cerevisiae* furnishing the best results. Competing reactions have been observed, however. Hamdani et al.[14] first reported a substrate-concentration-dependent competing dehalogenation (≤40%) to give **5**. The reaction conditions were not further specified, however, and the reaction has not been further investigated (Scheme 2).



Scheme 2. *Dehalogenation is the major pathway in the biotransformation of ethyl 2-chloroacetoacetate (****1****) with* Saccharomyces cerevisiae.

In follow-up experiments by Cabon et al., targeted towards the synthesis of stereopure **2**, even complete dehalogenation was encountered. Again, the phenomenon was not further investigated, but the authors discussed a mechanism that

[a] *G. Jörg, Dr. M. Bertau*
Technische Universität Dresden, Institut für Biochemie
01062 Dresden (Germany)
Fax: (+49) 351-463-35506
E-mail: martin.bertau@chemie.tu-dresden.de

 DOI: 10.1002/cbic.200300760

involved a radical NAD species.[15–17] In attempts to improve the stereoselectivity of the microbial reduction by addition of ethyl chloroacetate (**7**),[18] a significant decrease in dehalogenation (by 95%) was observed.[19]

Reductive dehalogenations had also been encountered with other halogenated β-keto esters,[20, 21] but until now no mechanism has been presented that explains the observations satisfactorily.

Our studies on the cytotoxicity of the substrate indicated that the dehalogenation might be associated with glutathione (GSH) as a consequence of xenobiotic stress, since substrate **1** has high alkylating power.[22, 23] This was the impetus to investigate a potential participation of GSH and/or a glutathione S-transferase (GST) in this reaction.

GST-catalysis is a common pathway in microbial dehalogenations. To the best of our knowledge, however, it has been described for bacteria, but not for fungi. Furthermore, with the exception of few examples, such as dechlorinations mediated by tetrachlorohydroquinone dehalogenase,[24, 25] bacterial dehalogenations mostly furnish the corresponding hydrolysis products.[26] In contrast, the unusual dehalogenation reported in this contribution is *reductive*, proceeds *aerobically* and is catalysed by a *fungus*. In this paper we present the mechanism of this novel type of dehalogenation.

Results

In our experiments with *S. cerevisiae*, 48% dehalogenation of substrate **1** was observed. The α-chloro carbinol **2** (51%) was obtained with 92% *ee* and 21% *de*. Addition of **7** to the culture inhibited dehalogenation by 99%. In all cases it was shown that chloride was in fact released from **1**, and that **4** and **5** are not the products of alternative pathways. The same applies for ethyl chloroacetate (**7**). There were no chloride sources other than the halo-organic substrates themselves. Quantitative dehalogenation as reported by Cabon et al.[19] was not observed.

By time-resolved GC/MS studies it was shown experimentally for the first time that the dehalogenation precedes the reduction of the keto group. In accordance with earlier reports, it was shown that the carbinolic species **2** and **3**—and also their enantiomers—are not substrates for the dechlorination reaction (Scheme 2).[19]

There were no indications of any involvement of radical species in the dehalogenation. When the biotransformation was conducted in the presence of the free radical scavenger 1,3-dinitrobenzene,[17] there was no detectable effect on the extent of dehalogenation. The same applied for experiments with cell liquor, in which the dehalogenation reaction occurred irrespective of whether a radical scavenger was present.

There was no detectable participation of hydrolytic dehalogenation variants in any whole-cell experiments.[27, 28] This also applied to hydrolytic GSH-dependent variants as catalysed by, for example, dichloromethane dehalogenase.[29] Aerobiosis displayed no measurable effects on intracellular GSH levels, but a 1.4-fold increase in microbial dehalogenation.

Dehalogenation is mediated by glutathione (GSH)

When GSH was added to a potassium phosphate-buffered aqueous solution of **1** in vitro, dehalogenation occurred spontaneously. The release of chloride was complete after 20 min, but no dehalogenation product **4** was found unless a 50-fold excess of GSH had been added. Even then, **4** was detectable only in trace amounts (< 0.1%).

When, for purposes of comparison, an in vitro experiment was conducted in the presence of equine liver glutathione S-transferase (EC 2.5.1.18, GST), chloride release proceeded 1.3 times faster than in the uncatalysed reaction (510 nmol min^{-1}), but formation of **4** was not affected. Under physiological conditions, where the intracellular substrate concentration amounts to about 2 mmol L^{-1}, the uncatalysed liberation of chloride from the chloro-organic substrate proceeded at a rate of 390 nmol min^{-1}. This is 2.6 times faster than the rate of about 150 nmol min^{-1} typically reported for aliphatic substrates (Figure 1).[30]

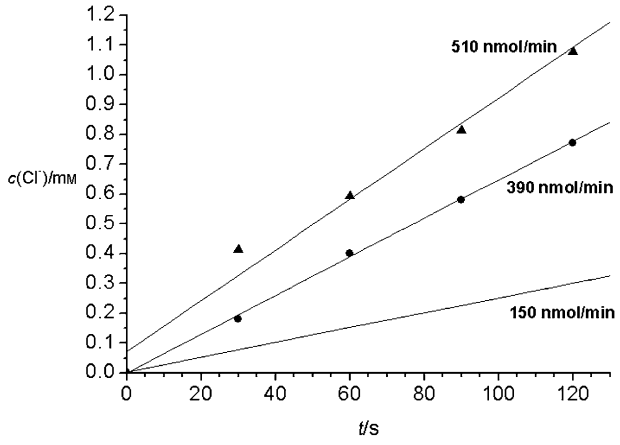


Figure 1. *Effects of enzyme catalysis on the release of inorganic chloride from the chloro-organic substrate **1** (-▲-: in vitro equine liver GST catalysis; -●-: abiotic; ——: literature value for chloroaliphatics).*

For whole-cell dehalogenation experiments, intracellular GSH concentration and GST activity were initially quantitated with 1-chloro-2,4-dinitrobenzene (CDNB) according to Habig's standard assay.[31] Under the applied conditions, the cytosolic GSH level was determined as 29 mmol L^{-1}, which is significantly elevated in relation to the concentration of 1 – 11 mmol L^{-1} reported in the literature.[32] Physiological GST activity was determined as 330 u L^{-1} cell liquor. Hence, the cells exhibit considerable reducing power. GST has only a minor catalysing effect on the release of chloride.

NMR, UV/VIS and LC/MS analyses of crude reaction mixtures allowed the unambiguous identification of glutathione conjugate **8** (Figure 2) as the central intermediate in this microbial dehalogenation. The ESI mass spectrum of this compound exhibits a single peak at $m/z = 436$, the $[M+H]^+$ peak of ethyl 2-glutathionylacetoacetate (**8**). From ^{1}H NMR analysis it was evident that a chemically uniform compound had been formed that consisted of a glutathionyl substructure and an acetoacetic

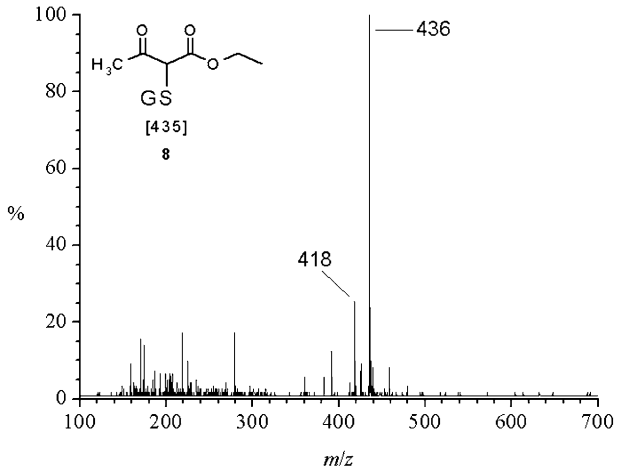


Figure 2. *Mass spectrometric evidence of the formation of glutathione conjugate* ***8*** *from substrate* ***1****.*

acid ethyl ester moiety with substitution at C-2. The ^{13}C NMR spectrum showed complete replacement of the C–Cl resonance at 94.6 ppm by a C–S resonance at 80.6 ppm. Under ambient conditions, intermediate **8** is stable for months both in the solid state and in aqueous solution. There are no indications of a reaction **8** → **4** other than under enzyme catalysis in vivo or in the presence of the 50-fold excess of GSH in vitro mentioned above. GC/MS analytical identification of ethyl 2-mercaptoacetoacetate and other sulfur-containing fragments supported these findings.

As a β-keto ester, conjugation product **8** exists in a tautomeric equilibrium with its enol (**9**) which, due to interactions with the glutathionyl moiety, establishes slowly within 16 h.

In a second step, conjugate **8** is transformed into **4**, accompanied by the formation of oxidised glutathione (GSSG). While conjugate formation also proceeds very fast abiotically, formation of dehalogenation product **4** apparently requires the action of an enzyme. As can be seen from Figure 3, enzyme catalysis keeps the concentration of intracellular **8** very low, due

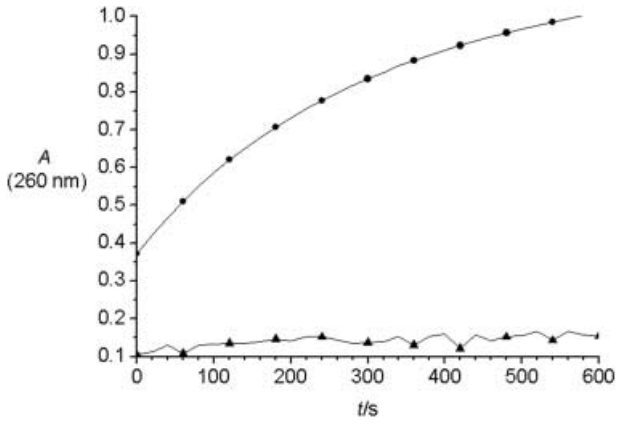
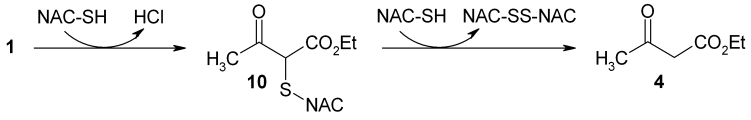


Figure 3. *Concentrations of* ***8*** *produced without catalysis (●) and with whole-cell catalysis (▲). Spectra were recorded at the maximum absorbance of the keto group ($\lambda_{max} = 260$ nm).*

to rapid transformation of the conjugate into **4**, while the conjugate remains unconverted in the absence of the biocatalyst.

The dehalogenating and deglutathionylating action of the thiol group was demonstrated in vitro with *N*-acetylcysteine (NAC) and 2-mercaptoethanol, each in 25-fold excess. In fact, substrate **1** was consumed completely, and dehalogenation via thioether intermediate **10** was accompanied by formation of NAC disulfide and 2-mercaptoethanol disulfide, respectively (Scheme 3).

When, on the other hand, the cells were thoroughly depleted of GSH by addition of *N*-ethylmaleimide (NEM)[33] neither dehalogenation of **1** nor deglutathionylation of **8** was observed; this clearly shows the involvement of GSH in this microbial reductive dehalogenation.



Scheme 3. *Treatment of* ***1*** *with the glutathione equivalent* N*-acetylcysteine (NAC) furnishes dehalogenation product* ***4*** *and oxidised NAC.*

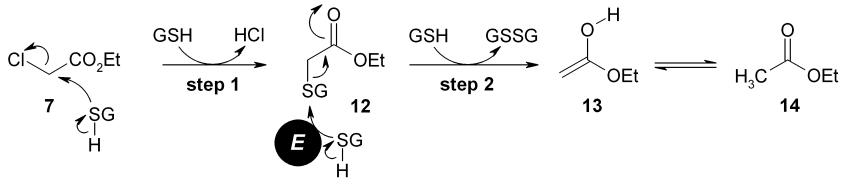
Inhibition studies

Ethyl chloroacetate (**7**) is reported to be a potent inhibitor of halo reduction. Cabon et al. point out that after preincubation with 15 mmol L^{-1} **7** for 3 h, competing dechlorination is reduced drastically, to a level of not more than 5%, while beforehand **5** had been the only observed reaction product.[19] In our own experiments with 15 mmol L^{-1} **7**, no dehalogenation product **5** was isolated. Although the inhibitory effect of **7** on the dechlorination reaction was not found to be as great, the results reflect a comparable trend. It is noteworthy that the same effect was also observed after preincubation with the substrate **1** itself.

To elucidate the inhibitory action of **7**, the compound was incubated with GSH in vitro. We found that GSH-mediated dechlorination of this species gave ethyl acetate (**14**), and that the reaction proceeded via ethyl glutathionylacetate (**12**), but 130 times more slowly than that of β-keto ester **1**. LC/MS analysis of the crude reaction mixture confirmed the formation of ethyl acetate (**14**) via thioether **12** and oxidised glutathione (GSSG) (Scheme 4).

Neither yeast nor equine liver glutathione transferase (EC 2.5.1.18) was inhibited by **7** or **1**, respectively. Instead, incubation of the cells with **7** or **1** prior to addition of substrate mostly depletes the cells of GSH, and after a 30 min incubation period with 15 mM **7**, intracellular GSH had dropped from 29 mmol L^{-1} to 0.3 mmol L^{-1}.

The dehalogenation phenomenon was found not to be restricted to respiro-fermenting cells (48%). We were able to show that this process is also active in anaerobically fermenting yeast cells (34%) as well as in resting cells (39%). Cell liquor also



Scheme 4. *The α-chloro ester **7** inhibits dehalogenation of **1** due to depletion of intracellular glutathione, and the inhibitor itself is dehalogenated slowly by the same mechanism as for **1**.*

showed dehalogenating activity (35%). These results emphasise the pivotal role of GSH.

Discussion

Our investigations were aimed at elucidation of the mechanism of the microbial reduction of **1** and the reason for the inhibitory action of **7** towards the dehalogenation reaction.

Broad knowledge of *an*oxic dehalogenations exists, mostly as bacterial, but also as fungal processes. None of these, however, is suitable to explain our findings, especially since this reductive dehalogenation is most effective under aerobic conditions.[24, 34–46] In fact, mechanisms involving single-electron transfer (SET), which would be well suited to explain our findings, have been reported to work exclusively under anoxic conditions.[15–17, 47, 48] Furthermore, involvement of these mechanisms had been excluded by running the biotransformation in the presence of the free radical scavenger 1,3-dinitrobenzene.

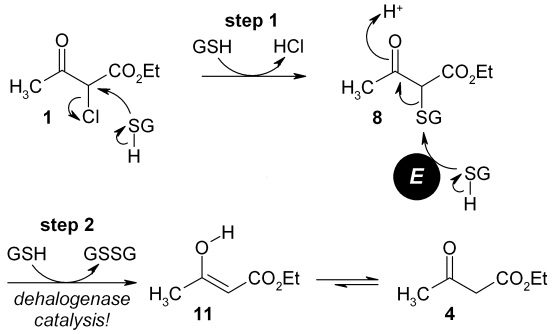
We have demonstrated that reductive dehalogenation of **1** is attributable to the high alkylating power of the substrate, as a consequence of which there is a fast reaction with the cell stress protectant glutathione. Our in vitro and in vivo investigations have for the first time allowed identification of the intermediate thioether **8**, formed by nucleophilic substitution of the chloro substituent by GSH. Hence, the active role of GSH in the dehalogenation of **1** is obvious.

Mechanistic model for the microbial dehalogenation.

Mechanistically, the microbial dehalogenation reaction is divided into two steps, the first of which does not necessarily require enzyme catalysis. In fact, the nucleophilic replacement of chloride by GSH proceeds mainly abiotically and is so fast that the 1.3-fold accelerating catalytic effect of a glutathione S-transferase (GST) is negligible. Chloride was liberated quantitatively from equimolar mixtures of **1** and GSH within minutes. The observed 1.3-fold rate enhancement for the glutathionylation of **1** with enzyme catalysis is still in good agreement with reported values for aliphatic substrates, which range between 1.05 and 2.4%.[49] The rather minor acceleration effect of GST is typical for aliphatic organohalogens and an expression of the broad substrate specificities of GST, which is due to their action in cellular detoxification processes. In contrast, aromatic substrates are far more easily accessible to GST catalysis.[23]

From our in vitro studies with GSH and GSH/GST we knew that no reductive dehalogenation occurred unless a 50-fold excess of GSH was applied. Even then, dehalogenation product **4** was detectable merely in trace amounts (< 0.1%), a clear indication of enzyme catalysis being required for the second half-reaction. Furthermore, as conjugate **8** is stable for months, alternate pathways for the production of **4** were excluded.

For the release of the dehalogenation product from **8** the presence of an α-chloro-positioned enolisable carbonyl group emerged as a strict prerequisite for the process to occur. After the reaction has been initiated by nucleophilic attack of GSH at C-2, accompanied by release of chloride, the intermediary thioether **8** is then attacked by a second species GSH. At this point the enolisability of the ketone becomes essential, as only an enolisable α-thioalkyl ketone substructure, as in **8**, would allow the attack of a second species GSH, in the course of which two electrons are transferred to the substrate. The negative charge is accepted by the carbonyl oxygen, furnishing **11**. Hence the enolisable α-thioalkyl ketone substructure serves as an essential electron sink. The final product of GSH-mediated thiolysis of the intermediary thioether **8** is **4**. Under physiological conditions this process is strictly enzyme-catalysed, by an as yet unidentified glutathione-dependent enzyme (**E**), and consumes GSH in doubly stoichiometric amounts (Scheme 5).



Scheme 5. *Mechanism of glutathione-dependent fungal reductive dehalogenation mediated by* Saccharomyces cerevisiae.

Consequently, as there is no possibility to transfer two electrons to the substrate in non-enolisable substrates—that is, as an electron sink is missing—there is no option to form GSSG. Hence, the only pathway for non-enolisable glutathionyl thioethers to react is hydrolytically. This is exactly what is observed in the majority of all aliphatic dehalogenations.

From the mechanism, the reason why reductive dehalogenation must precede carbonyl reduction to **2** and **3** is now evident: the β-halohydrins are not accessible to dechlorination, because of the lack of enolisability. Next, as C-2 is less activated in the β-hydroxy esters **2** and **3** than in β-keto ester **1**, compounds **2** and **3** exhibit poor alkylating power, because of which physiological

conditions are too mild for nucleophilic substitution of chloride by GSH or water.

Inhibition studies

Experiments with ethyl chloroacetate (**7**) revealed the mode of action of the dehalogenation inhibitor. Treatment of **7** with GSH produces ethyl acetate (**14**) and glutathione disulfide (GSSG) via conjugation product **12**, leading to a substantial depletion of GSH (99%). Because of ester resonance, the subsequent reaction of **12** with a second species GSH is by far less favoured, as a result of which the reaction **7** $\rightarrow$**14** proceeds 130 times more slowly than the conversion of **1** with GSH (Scheme 4).

The remaining dehalogenation activity is the result of the action of free GSH formed in response to xenobiotic stress. For whole-cell biotransformations it is therefore reasonable to determine cytosolic GSH levels in the strain used, in accordance with which the inhibitor should be applied in order to avoid further cytotoxic effects.

Conclusion

We have shown that the halo reduction of **1** is mediated by glutathione, which is the most universal agent involved in cell stress response and cellular detoxification reactions. Reductive dechlorination by respiro-fermenting, fermenting and also resting *S. cerevisiae* can be fully understood by the action of a novel, so far unidentified glutathione-dependent dehalogenase.

To the best of our knowledge, in this contribution we have presented the first fungal aerobic reductive dehalogenation of an aliphatic substrate according to a novel type of glutathione-dependent mechanism.

Further studies will aim to isolate the glutathione-dependent dehalogenase and to elucidate contributions of xenobiotic cell stress responses to the phenomenon of dehalogenation. Next, the investigation of potential stereoselectivities of dehalogenations is a matter for subsequent studies.

This novel dehalogenation mechanism has great implications for the metabolic fates of α-halo ketones in eukaryotic cells.

Experimental Section

General: *Saccharomyces cerevisiae* L13 was a product from the Societé industrielle de levure FALA, Strasbourg, France. Ethyl 2-chloroacetoacetate (**1**) was purchased from Fluka, additives and organic solvents were purchased from Acros. Equine liver glutathione S-transferase was obtained from Sigma.

Product identity was confirmed by NMR spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ and D_2O with a Bruker DRX 500 spectrometer at 500 MHz. Chemical shifts are quoted in ppm from internal TMS or TSP. Fermentations were performed in a 15 L Infors HT ISF200 fermenter. GC/MS analyses were carried out on a Hewlett Packard GC 5890 series II instrument equipped with a Hewlett Packard 5965B infrared detector and a Hewlett Packard 5972 mass selective detector (EI, 70 eV). HPLC analyses were done with a LiChrospher® 100 RP-18 column on a Knauer Wellchrom system, Electrospray LC/MS analyses were carried out on a Perseptive Biosystems Mariner Biospectrometry Workstation with LiChrospher® 100 RP-18 column. The absolute configurations of the products were determined on a Carl Zeiss Jena polarimeter Polamat A ($c = 1.0$, $CHCl_3$) and by comparison of the obtained results with reference data in ref. [19]. pH values were determined with a Mettler Toledo pH 320 instrument. UV/VIS spectra were recorded on an Amersham Pharmacia Biotech Ultrospec 3100 pro UV/visible spectrophotometer. All reactions were monitored by GC by use of an Analytik Jena Perichrom GC ST200 instrument.

Determination of extents of conversion: The conversions were measured by use of a J&W Scientific DB-5 column (30 m, 0.25 mm i.d.) at 50 °C (isothermal). The pressure of N_2 gas was 80 kPa and the temperatures of the injector and the detector were 210 °C and 260 °C, respectively. The keto-substrate **1** and products **2**, *ent*-**2**, **3** and *ent*-**3** were observed at retention times of 9.1 min, 9.4 min and 9.7 min, respectively. The % conversions were determined by use of an integrator.

Chloride assays: Time-resolved liberation of chloride from the chloro-organic substrate and chloride assays were conducted in accordance with ref. [50].

GSH/GST assays: GSH/GST assays were conducted according to the method of Habig et al.[31]

Whole-cell biotransformation of 1: Biotransformations were run on the 5 mL scale in a 15 mL Falcon tube. Conversion and yields were determined by GC and HPLC. The corresponding reactions on the 5 L scale in a 15 L fermenter gave identical results and isolated yields were determined as stated below. In all experiments the starting material was distilled directly before use in order to avoid potential effects by impurities.

Saccharomyces cerevisiae (Baker's yeast, 80 g dry weight) was suspended in a solution of sucrose (250 g) in tap water (5.0 L) in a 15 L fermenter. The aerobic culture was incubated at 30 °C and stirred for 30 min. Saturation with oxygen was kept above 20%. After continuous addition of **1** (50 mL, 59.05 g, 356 mmol) was complete (16 h), the suspension was stirred at 30 °C for 4 h. The biotransformation was monitored by GC. The reaction mixture was centrifuged, the yeast was washed with water, and the combined aqueous phase was extracted with diethyl ether (3×1.0 L). The organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. Distillation of the crude residue under reduced pressure yielded the carbinols **2** (18.62 g, 112 mmol, 31%), **3** (12.16 g, 73 mmol, 20%) and **5** (22.56 g, 171 mmol, 48%) as colourless liquids.

In vitro experiments: The in vitro experiments were performed with potassium phosphate buffered (pH 6.5) solutions of **1** (15 μL, 108 μmol) in 1.5 mL Eppendorf tubes. The reaction mixtures were shaken (500 rpm) at 30 °C for 2 h and analysed by GC/MS and LC/MS. Each experiment was paralleled by a blank experiment.

Ethyl 2-glutathionyl-acetoacetate (8): ^{1}H NMR (500 MHz, D_2O): $\delta =$ 4.19 (q, OCH_2CH_3), 4.18 (m, H_2NCH), 3.79 (m, SCH'_2), 3.25 – 2.70 (m, 2H'), 2.48 (m, CH'_2), 2.33 (s, CH_3), 2.31 (s, CH_3), 2.09 (m, CH'_2), 1.20 (t, OCH_2CH_3) ppm; ^{13}C NMR (500 MHz, D_2O): $\delta = 203.3$ (C=O), 203.3 (C=O), 177.3, 176.9, 176.1 ($C'ONH_2$), 173.8 ($C'ONH_2$), 171.2 ($C'O_2H$), 169.1 ($C'O_2H$), 169.1 (CO_2Et), 94.6 (C–OH, enol), 80.6 (C–S), 63.6 (OCH_2CH_3), 62.4 (OCH_2CH_3, enol), 54.0 ,52.7, 52.6, 43.3, 32.6, 32.5, 31.3, 27.7, 27.6 (CH_3), 26.1 (CH_3, enol), 13.2 (OCH_2CH_3) ppm; MS (ESI, 80 eV): *m/z* (%): 436 (100) $[M+H]^+$, 418 (25) $[M-H_2O]^+$.

Acknowledgements

We gratefully thank Prof. Dr. K.-H. van Pée for discussions, encouragement and support, and also acknowledge financial support by the Graduate Research Centre "Heterocycles" and the German National Merit Foundation. We thank M. Pitsch and B. Grzelak for technical assistance, Dr. U. Schwarzenbolz for LC/MS analyses, Dr. H. Kroschwitz for GC/MS analyses, and Dr. M. Gruner and A. Rudolph for recording NMR spectra.

Keywords: biotransformations · dehalogenases · glutathione · metabolism · redox chemistry

[1] O. A. Phillips, E. E. Udo, A. A. M. Ali, N. Al-Hassawi, *Bioorg. Med. Chem.* **2003**, *11*, 35 – 41.
[2] S. C. Bergmeier, S. J. Katz, *J. Comb. Chem.* **2002**, *4*, 162 – 166.
[3] G. E. Zurenko, J. K. Gibson, D. L. Shinabarger, P. A. Aristoff, C. W. Ford, W. G. Tarpley, *Curr. Opin. Pharmacol.* **2001**, *1*, 470 – 476.
[4] B. H. Yagi, G. E. Zurenko, *Anaerobe* **1997**, *3*, 301 – 306.
[5] S. Götzö, D. Seebach, J.-J. Sanglier, *Eur. J. Org. Chem.* **1999**, 2533 – 2544.
[6] F. Coelho, R. C. Rossi, *Tetrahedron Lett.* **2002**, *43*, 2797 – 2800.
[7] C. Yu, Y. Jiang, B. Liu, L. Hu, *Tetrahedron Lett.* **2001**, *42*, 1449 – 1452.
[8] X. Rabasseda, L. A. Sorbera, J. Castaner, *Drugs Future* **1999**, *24*, 1057 – 1067.
[9] A. Tixidre, L. Zard, G. Rossey, A. Bourbon (Synthelabo), EP0736606, **1996**.
[10] D. J. Ager, I. Prakash, D. R. Schaad, *Aldrichim. Acta* **1997**, *30*, 3 – 12.
[11] T. Nishiyama, S. Matsui, F. Yamada, *J. Heterocycl. Chem.* **1986**, *23*, 1427 – 1429.
[12] F. Benedetti, S. Norbedo, *Tetrahedron Lett.* **2000**, *41*, 10071 – 10074.
[13] M. Bertau, M. Bürli, E. Hungerbühler, P. Wagner, *Tetrahedron: Asymmetry* **2001**, *12*, 2103 – 2107.
[14] M. Hamdani, B. De Jeso, H. Deleuze, A. Saux, B. Maillard, *Tetrahedron: Asymmetry* **1993**, *4*, 1233 – 1236.
[15] L. M. Aleixo, M. de Carvalho, P. J. S. Moran, J. A. R. Rodrigues, *Bioorg. Med. Chem. Lett.* **1993**, 3, 1637 – 1642.
[16] M. de Carvalho, M. T. Okamoto, P. J. S. Moran, J. A. R. Rodrigues, *Tetrahedron* **1991**, *47*, 2073 – 2080.
[17] D. D. Tanner, A. R. Stein, *J. Org. Chem.* **1988**, *53*, 1642 – 1646.
[18] K. Nakamura, Y. Kawai, A. Ohno, *Tetrahedron Lett.* **1990**, *31*, 267 – 270.
[19] O. Cabon, D. Buisson, M. Larcheveque, R. Azerad, *Tetrahedron: Asymmetry* **1995**, 6, 2199 – 2210.
[20] M. Bertau, *Tetrahedron Lett.* **2001**, *42*, 1267 – 1268.
[21] M. Bertau, *Biocatal. Biotransform.* **2002**, *20*, 363 – 367.
[22] G. Jörg, Dissertation, Technical University of Dresden (Germany), in preparation.
[23] M. Penninckx, *Enzyme Microb. Technol.* **2000**, *26*, 737 – 742.
[24] S. D. Copley, *Curr. Opin. Chem. Biol.* **2003**, *7*, 265 – 272.
[25] P. M. Kiefer, D. L. McCarthy, S. D. Copley, *Biochemistry* **2002**, *41*, 1308 – 1314.
[26] D. Gisi, T. Leisinger, S. Vuilleumier, *Arch. Toxicol.* **1999**, *73*, 71 – 79.
[27] T. Nakamura, T. Nagasawa, F. Yu, I. Watanabe, H. Yamada, *Biochem. Biophys. Res. Comm.* **1991**, **180**, 124 – 130.
[28] J.-Q. Liu, T. Kurihara, S. Ichiyama, M. Miyagi, S. Tsunasawa, H. Kawasaki, K. Soda, N. Esaki, *J. Biol. Chem.* **1998**, *273*, 30897 – 30902.
[29] D. Gisi, L. Willi, H. Traber, T. Leisinger, S. Vuilleumier, *Appl. Environ. Microbiol.* **1998**, 1194 – 1202.
[30] D. R. Dohnt, A. J. Quebbemann, R. F. Borch, M. W. Anders, *Biochemistry* **1985**, *24*, 5137 – 5143.
[31] W. H. Habig, W. B. Jakoby, *Methods Enzymol.* **1981**, *77*, 398 – 405.
[32] F. Q. Schäfer, G. R. Büttner, *Free Radical Biol. Med.* **2001**, *30*, 1191 – 1212.
[33] J. Datta, T. B. Samanta, *Mol. Cell. Biochem.* **1992**, *118*, 31 – 38.
[34] M. Bunge, L. Adrian, A. Kraus, M. Opel, W. G. Lorenz, J. R. Andressen, H. Görisch, U. Lechner, *Nature* **2003**, *421*, 357 – 360.
[35] K. Furukawa, *Trends Biotechnol.* **2003**, *21*, 187 – 190.
[36] D. B. Janssen, J. E. Oppentocht, G. J. Poelarends, *Curr. Opin. Biotechnol.* **2001**, *12*, 254 – 258.
[37] K. Anandarajah, P. M. Kiefer, B. S. Donohoe, S. D. Copley, *Biochemistry* **2000**, *39*, 5303 – 5311.
[38] H. De Wever, J. R. Cole, M. R. Fettig, D. A. Hogan, J. M. Tiedje, *Appl. Env. Microbiol.* **2000**, *66*, 2297 – 2301.
[39] T. Kurihara, N. Esaki, K. Soda, *J. Mol. Catal. B: Enzym.* **2000**, *10*, 57 – 65.
[40] C. Holliger, G. Wohlfarth, G. Diekert, *FEMS Microbiol. Rev.* **1999**, *22*, 383 – 398.
[41] S. Fetzner, *Appl. Microbiol. Biotechnol.* **1998**, *50*, 633 – 657.
[42] F. J. M. Verhagen, H. J. Swarts, J. B. P. A. Wijnberg, J. A. Field, *Appl. Env. Microbiol.* **1998**, *64*, 3225 – 3231.
[43] T. Leisinger, *Curr. Opin. Biotechnol.* **1996**, *7*, 295 – 300.
[44] J. Dolfing, J. E. M. Beurskens, *Adv. Microb. Ecol.* **1995**, *14*, 143 – 206.
[45] S. Fetzner, F. Lingens, *Microbiol. Rev.* **1994**, 641 – 685.
[46] S. Belkin, *Biodegradation* **1992**, *3*, 299 – 313.
[47] J. He, K. M. Ritalahti, K.-L. Yang, S. S. Koenigsberg, F. E. Löffler, *Nature* **2003**, *424*, 62 – 65.
[48] M. E. Walsh, P. Kyritsis, N. A. J. Eady, H. A. O. Hill, L.-L. Wong, *Eur. J. Biochem.* **2000**, *267*, 5815 – 5820.
[49] L. Scarponi, P. Perucci, L. Martinetti, *J. Agric. Food Chem.* **1991**, *39*, 2010 – 2013.
[50] M. Bertau, G. Jörg, *Anal. Biochem.* unpublished results.

Received: September 4, 2003 [F 760]

