Thiel, Daniel; Doknic, Diana; Deska, Jan

Enzymatic aerobic ring rearrangement of optically active furylcarbinols

Published in:
Nature Communications

DOI:
10.1038/ncomms6278

Published: 01/10/2014

Please cite the original version:
Enzymatic aerobic ring rearrangement of optically active furylcarbinols

Daniel Thiel¹, Diana Doknić¹ & Jan Deska¹

Biogenic furans are currently discussed as highly attractive alternative feedstock in a post-fossil society; thus, also the creation of sustainable furan valorization pathways appears of great importance. Here an artificial Achmatowicz monooxygenase activity for the aerobic ring expansion of furans is achieved by the combination of commercial glucose oxidase as oxygen-activating biocatalyst and wild-type chloroperoxidase as oxygen-transfer mediator, providing a biological ready-to-use solution for this truly synthetic furan rearrangement. In concert with enzymatic transformations for the enantioselective preparation of optically active furylcarbinols, purely biocatalytic reaction cascades for the stereocontrolled construction of complex pyranones are obtained, exhibiting high functional group tolerance even to oxidation-sensitive moieties.

¹Department für Chemie, Universität zu Köln, Greinstrasse 4, 50939 Cologne, Germany. Correspondence and requests for materials should be addressed to J.D. (email: jan.deska@uni-koeln.de).
In the course of the inevitable medium- to long-term reorganization of our value chains from fossil resources towards a general supply on the basis of renewable raw materials, one of the great challenges in the near future will comprise the development of novel processes and the adaptation of existing synthetic strategies to those new requirements. Currently, biogenic furans, obtained through biorefinery of lignocellulose, are considered one of the most promising core structures among the plant-based, non-edible raw material platforms. While the energetic use of renewables has to be met with a certain scepticism not only from a chemist’s perspective, the role of biomolecules as rich source of green synthetic building blocks is far less controversially discussed. In addition to defunctionalization routes from biogenic furans to synthetic building blocks is far less controversially discussed. In piperidine derivatives like transformations in biosynthesis, studies on the furan meta- and the desired pyranone could be isolated in 51% yield. In the light of a sustainable utilization of biogenic building blocks, the development of environmentally benign valorization pathways has to be regarded a paramount task, and in our opinion, biological catalysts have to be considered most suitable to fulfill these requirements. However, biocatalysis is hampered by one simple factor, that is, the lack of biosynthetic precedence for a variety of synthetically relevant transformations and the consequent lack of natural catalysts to promote the desired reactions. Thus, the identification of promiscuous activities by wild-type enzymes as well as the evolution of new reactivities with the aid of biotechnological and chemical tools are highly warranted.

In this report, we disclose an unprecedented, purely enzymatic protocol for the non-natural Achmatowicz-type rearrangement of furylcarbinols employing an oxygen activation cascade based on commercially available biocatalysts (Fig. 1b). Our experimental design rests on the initial finding of selective oxygen-transfer abilities of peroxidases in the oxidative ring expansion of substituted furylcarbinols. The linkage with a hydrogen peroxide-evolving system based on the bioreduction of aerial oxygen by cheap and ecologically benign biomolecules such as glucose renders a fully integrated, highly practicable and sustainable protocol allowing for the rearrangement not only of simple carbinols but likewise for a series of real-world substrates carrying synthetically versatile functional groups.

Results
Catalyst identification. Owing to the absence of Achmatowicz-like transformations in biosynthesis, studies on the furan metabolism in mammals served as foundation for our development of a synthetic enzyme-based protocol. Toxikokinetic studies on pulegone and menthofuran detoxification pathways by Nelson and co-workers did not only identify human liver cytochrome P450 monooxygenases as catalysts involved in furan degradation, but also provided evidence for the formation of furanepoxides, which is also expected in the chemical Achmatowicz oxidation by electrophilic oxygenation agents. Thus, as robust and relatively cheap functional mimics of cytochrome P450, four haem-based peroxidases were evaluated on their potential to catalyse the selective oxidation of furylcarbinol rac-1a to give the ring-expanded product rac-2a. With gluconate as sole carbon-based side product, commercial glucose oxidase (Aspergillus niger, GOx) was chosen as a release system for hydrogen peroxide through the catalytic reduction of aerial oxygen, offering the benefits of simple product separation and an excellent ecological profile.

In the absence of potential oxygen-transfer catalysts as well as in the presence of soybean peroxidase (Table 1, entry 1), no conversion was observed and rac-1a was recovered quantitatively after 2 days of incubation. In contrast, using bovine lactoperoxidase or horseradish peroxidase (Table 1, entries 2 and 3), slow consumption of the furylcarbinol was detected and in the latter case, traces of the Achmatowicz product rac-2a were isolated after 48 h. An entirely different picture was drawn implementing chloroperoxidase from Caldariomyces fumago (CPO) into the system. Sometimes referred to as peroxidase–cytochrome P450 functional hybrid, CPO shares structural properties with P450 enzymes such as a thiolate as axial haem ligand explaining a much more diverse reaction portfolio compared with other peroxidases including effective cytochrome-like oxygen-transfer processes. Within 2 h, full conversion of rac-1a was achieved and the desired pyranone rac-2a could be isolated in 51% yield.

As pH variation did not result in any improvement of the catalytic system (Supplementary Fig. 1), we turned our attention to potentially beneficial effects of cosolvents (Supplementary Fig. 2). Here, polar additives (10% v/v) like dimethylsulfoxide, acetone (Table 1, entries 5 and 6) or...
acetone reduced the rates of oxidation. On the other hand, in two-phase systems based on hydrophobic solvents (toluene, heptane, ethyl acetate) the oxidative ring expansion did not take place at all. In strong contrast, in the presence of tert-butanol as cosolvent, not only the enzymes’ activity was well conserved but more importantly, product rac-2a could be isolated in substantially higher yield of 82% (Table 1, entry 7). While the addition of tBuOH had only marginal effect on the rate of furan consumption, the boost in yield can rather be attributed to considerably enhanced product stability with a half-life of pyranone 2a of 40.7 h (± 3.7 h) as compared with 11.9 h (± 0.2 h) in the purely aqueous medium (Supplementary Fig. 3). In addition, we could prove that also other hydrogen peroxide-evolving systems such as l-leucine/l-amino-acid oxidase (Crotalus atrox) support the CPO-catalysed rearrangement; however, due to the significantly higher cost of the enzymes and lower reaction rates, these alternatives were not further pursued (Supplementary Fig. 4). Moreover, a single-enzyme approach was studied where hydrogen peroxide was slowly added by syringe pump to a buffered solution of rac-1a and CPO. In this case, full conversion was achieved after 2 h and rac-2a could be isolated in 67% yield (Table 1, entry 8).

**Mechanistic considerations.** With the identification of the GOx/chloroperoxidase couple as efficient system for the aerobic Achmatowicz oxidation, questions about the underlying functional principle arose. As the native functions of haloperoxidases include the oxidation of halides to hypohalites, the initial goal was to shed light on whether a halide/hypohalite mediator system qualified as final oxidizing species or rearrangement was induced through direct substrate–enzyme interaction and oxygen transfer from the prosthetic oxidized haem unit (compound I). First studies revealed that incubation of rac-1a in a buffered NaOCl solution in the absence of potential catalysts led indeed to oxidative ring expansion to give traces of pyranone 2a; however, reaction rates substantially lagged behind the GOx/CPO-catalysed transformation. Furthermore, considerable inhibition of the enzymatic rearrangement was observed in chloride-containing reaction media (half-maximal inhibitory concentration = 7.4 mM; Supplementary Fig. 5). Another obvious evidence for a direct interaction between substrate and chloroperoxidase resulted from the time-dependent stereochemical analysis of the GOx/CPO-mediated reaction of rac-1a.

**Table 1 | Oxidase/peroxidase-mediated oxidation of rac-1-(2-furyl)ethanol.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Peroxidase</th>
<th>Additive*</th>
<th>Time (h)</th>
<th>Conversion (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soybean peroxidase</td>
<td>—</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Lactoperoxidase</td>
<td>—</td>
<td>48</td>
<td>12 (−)d</td>
</tr>
<tr>
<td>3</td>
<td>Horseradish peroxidase</td>
<td>—</td>
<td>48</td>
<td>16 (&lt;5)</td>
</tr>
<tr>
<td>4</td>
<td>Chloroperoxidase C. fumago</td>
<td>2</td>
<td>&gt;99 (51)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Chloroperoxidase C. fumago</td>
<td>DMSO</td>
<td>2</td>
<td>44</td>
</tr>
<tr>
<td>6</td>
<td>Chloroperoxidase C. fumago</td>
<td>MeCN</td>
<td>2</td>
<td>58</td>
</tr>
<tr>
<td>7</td>
<td>Chloroperoxidase C. fumago</td>
<td>t-BuOH</td>
<td>2</td>
<td>&gt;99 (82)</td>
</tr>
<tr>
<td>8a</td>
<td>Chloroperoxidase C. fumago</td>
<td>t-BuOH</td>
<td>2</td>
<td>&gt;99 (67)</td>
</tr>
</tbody>
</table>

DMSO, dimethylsulphoxide.

Reaction conditions: rac-1a (10 mM), D-glucose (D-Glc, 50 mM), glucose oxidase (3.6 U), citrate buffer (pH 5.5), 30°C, 500 r.p.m.; conversions determined by gas chromatography using bromobenzene as standard.

a 10 v%.
b Isolated yield (2a) in parentheses, 0.5 mmol scale.
c Slow addition of aq. H2O2 instead of D-Glc/GOx.

d**Figure 2 | Indications for the direct peroxidase–furan interaction.** (a) Conversion of racemic furylmethanol by chloroperoxidase is accompanied by enrichment of the (S)-alcohol through kinetic resolution (kcat = 4.5). (b) Incorporation of 18O by incubation under 18O2-enriched argon atmosphere. (c) Coincubation of furylhydroperoxide and furylcarbinol in the absence of external oxidizing agents leads to selective formation of the hydroperoxide-derived product. The reaction was stopped at low conversion to deliberately overestimate 1a/1a-d3-scrambling as expected for a potential alcohol diffusion pathway.

**Table 2 | Enantiodiscrimination and oxygen labeling.**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Product</th>
<th>Conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air d-glucose GOX + CPO</td>
<td>rac-1a</td>
<td>41%</td>
</tr>
<tr>
<td>18O2 (20% in Ar) d-glucose GOX + CPO</td>
<td>rac-3a</td>
<td>58% 31% 18O</td>
</tr>
<tr>
<td>Ar-atmosphere CPO</td>
<td>rac-1a-d3</td>
<td>95% : 5 (53% ee)</td>
</tr>
</tbody>
</table>

Kinetic resolution: E = 4.5

High isotopic yield

Coupled deoxygenation/oxygenation
At low conversion, a distinct kinetic resolution with an enantioselectivity of $E = 4.5$ was observed (Fig. 2a), implying at least the participation of a protein-bound oxidizing species. The strongest indication for the non-mediated furan oxidation through compound I, or alternatively through a hydroperoxoferric intermediate, arose from experiments using isotopically labelled oxygen as terminal oxidant. Ring expansion by the GOx/CPO system in an argon atmosphere enriched in heavy oxygen (20 vol% $^{18}$O$_2$) gave rise to the mono-$^{18}$O-marked product rac-$2a$ in high isotopic yield (Fig. 2b). Moreover, mass spectrometric fragment analysis disclosed a low degree of regioselectivity in the oxygen transfer onto the furan core with a slight preference for the less-substituted position (Supplementary Fig. 6). Determination of the kinetic parameters for the aerobic oxidation of rac-$1a$ revealed an apparent turnover frequency of the peroxidase of $k_{\text{cat}} = 370.7 \text{ min}^{-1}$ ($\pm 9.3 \text{ min}^{-1}$) and a good affinity for this non-natural substrate of $K_{\text{M}} = 141 \mu \text{M}$ ($\pm 27 \mu \text{M}$) with a total turnover number of $\sim 121,400$ at 50 mM substrate concentration (Supplementary Fig. 7). In addition, the hypothesis of oxygen transfer from the oxidized prosthetic group was strongly supported by fundamental experiments on a redox-neutral enzymatic rearrangement. Oxygen-transfer-based redox-isomerization of hydroperoxide rac-$3a$ by chloroperoxidase from C. fumago as sole catalyst did not only proceed smoothly, coinubcation with equimolar amounts of the deuterium-labelled alcohol rac-$1a$-$d$, yielded the Achmatowicz product rac-$2a$ alongside with only minor amounts of the $CD_3$-marked pyranone rac-$2a$-$d$. While indicating that initial reduction of the hydroperoxide and reoxidation of the so-formed furylcarbinol take place in a coupled manner without diffusion as dominant pathway (Fig. 2c; Supplementary Fig. 8), this experiment also clearly refutes working hypotheses about non-haem or ferric oxidizing species. Moreover, also the use of $t$-butyl hydroperoxide as external oxidizing agent proved to be effective ($k_{\text{cat}} = 98.8 \text{ min}^{-1}$ ($\pm 28.1 \text{ min}^{-1}$)) and led to the formation of rac-$2a$ from alcohol rac-$1a$ in the presence of chloroperoxidase, ruling out alternative reaction pathways such as formation and consequent reduction of endoperoxide intermediates. On the basis of these results, we propose that the biocatalytic Achmatowicz-type rearrangement by chloroperoxidase proceeds via oxygenation of the furan core through oxygen transfer from an oxoferryl porphyrin species and subsequent ring fission and recycylation to give the pyranone products (Supplementary Fig. 9).

Towards coupled multi-enzymatic processes. Even if a certain extent of stereoselectivity was observed in the oxidative transformation of rac-$1a$, the low degree of enantiomeric discrimination would not allow for a preparatively reasonable kinetic resolution of this substrate. As kinetic resolutions exhibit the general flaw of yield limitation, our aim was to synthesize optically active furylcarbinols via preceding biotransformations to subsequently rearrange them on the basis of the artificial Achmatowicz monoxygenase system. The asymmetric reduction of the corresponding furylketones (3a-k) through alcohol dehydrogenases (ADHs) proved to be an efficient and convenient method. Two commercial ADHs in a substrate-coupled system with isopropanol as terminal reducing agent enabled the preparation of alcohol rac-$1a$ whereby, depending on the employed ADH, both (R)-$1a$ and (S)-$1a$ were obtained in nearly enantiopure form. The succeeding GOx/CPO-mediated aerobic rearrangement to the pyranones (R)-$2a$ and (S)-$2a$, respectively, proceeded in good yield, with quantitative preservation of optical activity and

| Table 2 | Biocatalytic Achmatowicz rearrangement of optically active furanals. |

Asymmetric ketone reduction (alcohol dehydrogenase) or kinetic alcohol resolution (lipase) → Air, D-glucose oxidase (A. niger) chloroperoxidase (C. fumago) → Citrate buffer (pH 5.5) iBuOH, r.t., 2 h

<table>
<thead>
<tr>
<th>Reaction conditions: α-glucose (Glc; 50 mM), furylcarbinol 1 (10 mM), GOx (3.6 μM, 0.5 kU), CPO (0.41 μM, 0.5 kU), citrate buffer (45 ml, 0.1 M, pH 5.5), iBuOH (5 ml), room temperature (RT), 500 r.p.m.; isolated yields after column chromatography.</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPrelog-selective ADH was used for the synthesis of (S)-$1a$.</td>
</tr>
<tr>
<td>bAbsolute configuration established independently according to Prelog’s rule (anti-Prelog-selective ADH) and Kazarlaskas’ rule in the acyclic lipase-catalysed kinetic resolution. See Supplementary Methods for full experimental details.</td>
</tr>
</tbody>
</table>

MO, methoxymethyl.
Figure 3 | Combination of two glucose-dependent biotransformations: four enzymes—two steps—one pot. The reaction was performed in a sequential manner due to incompatible pH optima of the individual catalytic systems. See Supplementary Methods for full experimental details.

Figure 4 | Aerobic Achmatowicz oxidation of 2,5-disubstituted furans. On the basis of the enzyme cascade consisting of an oxidase and a lipase, peracetic acid is formed catalytically from the acetate buffer that can act as diffusible redox mediator in the oxidation of otherwise non-reactive furylcarbinols.

without perceptible matched/mismatched effects (Table 2). Employing the anti-Prelog-selective ADH, various ketones were reduced in high enantioselectivity and subsequently subjected to the aerobic biocatalytic rearrangement. Oxidative ring expansion of the enantiomERICALLY enriched ethyl- and propyl-substituted alcohols 1b and 1c and even of the sterically encumbered isopropylcarbinol 1d succeeded in good yield. Moreover, the CPO-catalysed transformation of the alcohol 1c (95% ee) and 1d (97% ee) was accompanied by further increase in optical purity of the pyranone products (≥98% ee). The extent of enantio-discrimination of CPO towards substrate 1d was determined in an oxidative kinetic resolution with a relative rate of $k_{95}/k_{97} = 9.6$. Hence, with regard to a broader substrate tolerance in both enantiomeric series, evolution of less-stereoselective chloroperoxidases appears desirable in the longer run. Apart from that, a high functional group tolerance of CPO could be illustrated in the rearrangement of the alcohols 1e-1h. Not only substrates such as chlorohydrine 1e or β-hydroxyester 1f smoothly rearranged in the presence of the GOx/CPO system to give the pyranones 2e (64%) and 2f (68%), respectively, more gratifyingly, even oxidation-sensitive elements like olefins (2g, 65%) and primary alcohols (2h, 31%) were well tolerated by the peroxidase. Solely, oxidation of the propargylic alcohol 1i turned out problematic and the corresponding rearrangement product could not be isolated. With the use of core-substituted furylcarbinols, a structural limitation became apparent. While biocatalytic reduction of the 5-substituted furylketones gave rise to the enantiopure alcohols 1j and 1k, their oxidative ring expansion failed even with prolonged reaction time and substantially increased enzyme loadings.

Since both the asymmetric ketone reduction for the construction of enantioenriched furylcarbinols as well as their oxidative rearrangement could potentially be attained on the basis of glucose-dependent biotransformations, it seemed logical to strive after a direct linkage of both reactions to establish a multi-enzyme cascade for the synthesis of optically active Achmatowicz products. Essentially, pyranone (R)-2a could be detected in traces in a genuine four-enzyme/one-pot reaction; however, more satisfying results were obtained by sequential addition of the individual catalysts and concurrent pH adjustment. Thus, after reaching nearly full conversion of the ketone 4a by a glucose dehydrogenase (GDH)/ADH system, the reaction mixture was slightly acidified. With the addition of GOx and chloroperoxidase, fast and selective formation of the pyranone was observed (Supplementary Fig. 10) and (R)-2a was isolated in 47% yield and >99% ee (Fig. 3). In a triple catalytic system, where the GDH-mediated cofactor recycling was replaced by the previously employed dehydrogenation of isopropanol, the same one-pot principle provided (R)-2a in enantiopure form and 59% yield.

Diffusible redox mediators to overcome structural limitations. Although excellent selectivities, sustainable reaction conditions and high functional group tolerance of the presented Achmatowicz system leave no doubt that this novel application represents a valuable extension of the existing biocatalytic portfolio, our delight was marginally tempered by its limitation to mono-substituted furans. Directed evolution of the biocatalyst with regard to an extended substrate spectrum seems to be a logical next step to address this issue37. From the chemist’s perspective, however, also the reconstruction of the catalytic system’s architecture by allowing for non-protein-bound diffusible oxygen-transfer mediators appeared as an attractive alternative to eliminate undesired substrate effects. In search of suitable redox mediators combining activity in the furan oxidation with the disability to oxidize secondary alcohols, our choice fell on peracetic acid. Perhydrolysis of carboxylic acid derivatives through lipases and utilization of the so-formed peracids in oxidation reactions represents a well-studied approach38-39. In analogy to the oxidase/peroxidase couple, we aimed for the concatenation of the biocatalytic reduction of aerial oxygen with the activation of hydrogen peroxide by enzymatic peracetal formation. The combination of GOx with a lipase from Candida antarctica (type B, non-immobilized preparation) in acetate buffer at pH 4.5 proved to be a viable catalytic system for the selective aerobic oxidation of rac-1j, as the 2,6-dimethylated pyranone 2j was produced in moderate yield (Fig. 4). Even if substantially prolonged reaction times and the unfavourable optimum pH with regard to configurational lability of the furylcarbinols (see Supplementary Fig. 11, for racemization under acidic conditions) unquestionably demand vernier adjustments of this biocatalytic Achmatowicz variant, the application of diffusible, yet selective mediators might mature to an auspicious and practical alternative to the direct oxidation by haem-based enzymes.

Discussion

Over the years, biocatalysis has made its way into synthetic organic chemistry; however, in most cases, the role of enzymatic transformations is limited to the supply of non-racemic small-molecule building blocks with only marginal-to-non-existing contribution to the creation of molecular complexity. Herein, we demonstrated the use of biocatalysts outside their natural setting, tackling a non-natural rearrangement reaction converting structurally simple and readily available furylcarbinols into high-value synthetic building blocks in a preparatively convenient and eco-friendly manner. Aiming for the valorization of biogenic furans, future investigations will expand towards biological cascades for the direct conversion of lignocellulose-derived furfurs by means of lyases as well as the construction of combined organo-/biocatalytic reaction sequences. With the recent advances in biotechnological process development, there is a true potential to design fermentation pathways implementing further optimized peroxidases or monoxygenases for selective
Achmatowicz-type rearrangements that would allow for integrated furan refinement up the ladder of molecular complexity also in a more technical context.

Methods

General methods. Commercially available reagents were used without further purification. 13C (98 atom%) was purchased from CarboChemicals GmbH. Catalysts and cofactors were obtained from: NADH, CarboChemicals GmbH; chloroperoxidase from C. fumago (CPO), 21.9 kU ml−1, Bio-Research Product Laboratories; 2,2′-azobis(2-methylpropionitrile) (AIBN), 0.19 mmol, 47%, Sigma; ADH, recombinant from Escherichia coli (ADH 200), 17.6 U mg−1, evocatal GmbH; ADH, recombinant from E. coli (ADH 030), 18.8 U mg−1, evocatal GmbH; and glucose dehydrogenase, recombinant from E. coli (GDH 060), 214 U mg−1, evocatal GmbH. Presented data are average numbers of at least two replicate experiments. All products were purified by column chromatography over silica gel (Macherey-Nagel MN-Kieselgel 60 (40–63 µm, 240–400 mesh)). Analytical thin-layer chromatography was performed on Macherey-Nagel precoated silica gel plates (ALUGRAMM Sil G/UV254). 1H- and 13C-NMR spectra were recorded on a Bruker Avance 300 FTIR spectrometer, absorption bands were reported in wave numbers (cm−1).

Biocatalytic Achmatowicz rearrangement. In a 100 ml round-bottom flask, furfurylcarbinol (0.5 mmol) was dissolved in a mixture of citrate buffer (25 ml, 0.5 M, pH 5.5) and 2-butanol (5 ml). After addition of chloroperoxidase (0.41 µM, 0.5 kU/L) and 2-glucose (50 mM), the mixture was incubated for 24 h at 25 °C. High-performance liquid chromatography was performed on a Merck D-7000 with a Merck L-4500 PDA detector using an analytical Daicel Chiralpak column (250 × 4.6 mm). Optical measurements were reported on a Perkin-Elmer 343Plus. For full experimental details, see Supplementary Methods.

References

Acknowledgements

This research was financially supported by the Fonds der Chemischen Industrie (Liebig fellowship to J.D.) and the German Research Foundation (DFG: DE1599/4-1). Support by Bio-Research Products Inc. and Carbolution Chemicals GmbH is gratefully acknowledged.

Author contributions

D.T. and D.D. performed the experiments, compound characterization and data analysis and contributed to the experimental design. J.D. designed and supervised the project and wrote the manuscript.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Thiel, D. et al. Enzymatic aerobic ring rearrangement of optically active furylcarbinols. Nat. Commun. 5:5278 doi: 10.1038/ncomms6278 (2014).