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Access to Lactone Building Blocks via Horse Liver Alcohol Dehydrogenase-Catalyzed Oxidative Lactonization

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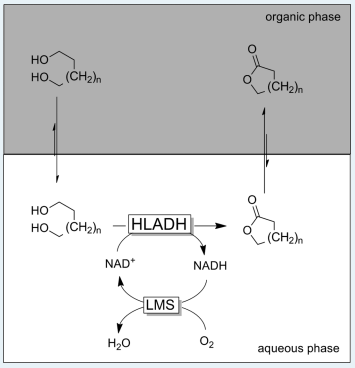
Supporting Information

ABSTRACT: The oxidative lactonization of 1,4-, 1,5-, and 1,6-diols using horse liver alcohol dehydrogenase (HLADH) is reported. Molecular oxygen was used as terminal electron acceptor by utilization of the laccase-mediator concept to regenerate the oxidized nicotinamide cofactor and producing water as sole byproduct. Spontaneous hydrolysis of the lactone products was identified as a major limiting factor toward preparative application of the system, which can be alleviated by using a two liquid phase approach to extracting the product into an organic solvent.



KEYWORDS: *lactones, alcohol dehydrogenase, laccase-mediator system, cofactor regeneration, two liquid phase systems, biocatalysis, chemoenzymatic cascades*

Lactones represent an important class of substances with applications not only in polymer synthesis[1] but also as environmentally benign solvents,[2] fuels,[3] and as building blocks for synthesis.[4]

Their preparation via esterification of hydroxy acids[5] or Baeyer–Villiger Oxidation of cyclic ketones[6] is well-documented in the literature.

Oxidative lactonization of diols represents an alternative route to lactones, but, though long-known, is far less explored.[7] Recently, we have applied the oxidative lactonization of 1,4-butanediol as a "smart cosubstrate approach" to render alcohol dehydrogenase (ADH)-catalyzed Meerwein–Ponndorf–Verley reductions irreversible.[8] Among other ADHs, horse-liver alcohol dehydrogenase (HLADH) excelled by its high activity toward 1,4-butanediol. Therefore, we became interested in further exploring the scope of the HLADH-catalyzed oxidative lactonization of diols. Particularly, the oxidative lactonization of some *meso*- and chiral diols was investigated with respect to the formation of enantiomerically enriched lactones. Furthermore, we aimed at raising the reactant concentrations to preparatively more relevant levels.

For regeneration of the oxidized nicotinamide cofactor (NAD^+) we chose the recently established laccase–mediator system (LMS) utilizing the robust laccase from *Myceliophthora thermophila* (*Mt*laccase) and acetosyringone as redox mediator.[9] Overall, an aerobic chemoenzymatic cascade for the oxidative lactonization of diols as outlined in Scheme 1 was envisioned.

In a first set of experiments we evaluated a range of 1,4-, 1,5-, and 1,6-diols (Table 1). 1,3-Diols were evaluated as well but did not yield detectable lactone formation, most probably because of the significant ring strain of these systems. It is also worth mentioning here that in the absence of any catalytic component (HLADH, *Mt*laccase, NAD^+ or mediator) no significant conversion was detected.

As shown in Table 1, 1,4- and 1,5-diols were converted smoothly by the HLADH–LMS system, whereas ε-caprolactone (**5b**) formation (from 1,6-hexanediol, **5a**) proceeded rather sluggishly, reaching only 26% conversion even after prolonged reaction times. The latter result is in apparent contrast to the spectrophotometrically determined activity of HLADH toward 1,6-hexanediol (see Supporting Information, Figure S2). However, it should be kept in mind here that this assay presumably only covers the first step of the lactonization cascade (i.e., HLADH-catalyzed formation of the aldehyde). It remains to be elucidated whether the slow accumulation of ε-caprolactone is due to poor activity of HLADH toward the 7-membered lactol, or if the lactol formation itself is rate-limiting. NMR analysis of the reaction mixtures, however, revealed no detectable aldehyde accumulation but indicated lactol accumulation at roughly the same level as the lactone product. At the same time, significant acidification of the reaction mixture was

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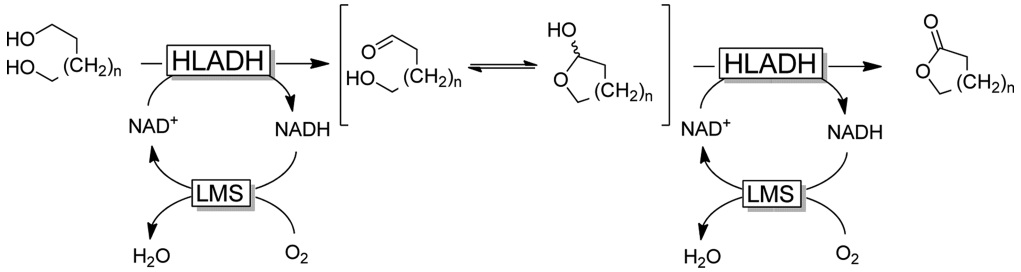
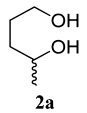
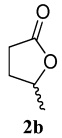
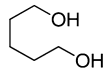
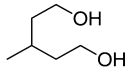
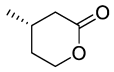
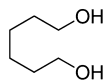
Scheme 1. Oxidative Lactonization of Diols to the Corresponding Lactones Catalyzed by Horse Liver Alcohol Dehydrogenase (HLADH)[a]



[a]Aerobic regeneration of the oxidized nicotinamide cofactor (NAD^+) is achieved by using the so-called laccase-mediator system (LMS) comprising the laccase from *Myceliopthora thermophila* (*Mt*laccase) and acetosyringone as redox mediator (the structure of acetosyringone and the proposed NADH-oxidation mechanism is shown in the Supporting Information, Figure S10). n = 1−3.

Table 1. HLADH−LMS-Mediated Oxidative Lactonization Reactions[a]

Diol	Lactone	k_{cat}/K_M [$s^{-1}M^{-1}$][b]	Conversion [%]	*ee* [%]	pH[c]
 **1a**	 **1b**	97	>99	-	n.d.
 **2a**	 **2b**	54	82	2	7.3
 **3a**	 **3b**	326	80	-	6.3
 **4a**	 **4b**	511	>99	>99 (*S*)	6.6
 **5a**	 **5b**	500	26	-	6.6

[a]Reaction conditions: c(diol) = 50 mM, $c(NAD^+)$ = 0.5 mM, c(HLADH) = 0.3 g L^{-1}, c(*Mt*laccase) = 2 μM, c(acetosyringone) = 0.2 mM, buffer: Tris-HCl (50 mM, pH 8), T = 30 °C, reaction time: 24 h. [b]Determined spectrophotometrically, see Supporting Information for full experimental details and results. [c]Determined after 24 h.

observed which may explain the rather poor product yield. Further investigations clarifying these issues are currently underway.

The oxidation reactions proceeded with high chemo- and regioselectivity. Occasionally, traces of the putative lactol intermediates were observable via GC/MS analysis, but all disappeared in the course of the reactions. No indications for the formation of dialdehydes (as dead-end products) could be found. Also, in case of 1,4-pentanediol (**2a**), no indications for the oxidation of the secondary OH-group (and formation of the hydroxyketone) could be found. Unfortunately, the enantiomeric purity of the corresponding lactone product (**2b**) was very low (maximally 17% ee) and decreased with increasing conversion of the starting material. Possibly, HLADH does not discriminate chiral centers in γ-position to the reacting OH-group as efficiently as in β-position leading to a poor kinetic resolution of the racemic **2a** (vide infra).

In contrast to the poor kinetic resolution of **2a**, the oxidative desymmetrization of 3-methyl-1,5-pentanediol (**4a**) proceeded smoothly with exquisite stereoselectivity (*S*), giving access to an interesting building block in the total synthesis of, for example, (*R*,*Z*)-5-muscenone, tulearin C, 7,20-diisocyanoadociane, neopeltolide macrolactone, and (*S*)-methanophenazine.[10]

Encouraged by these results, we evaluated the possibility of increasing the substrate concentration to more preparatively interesting values. Hence, we performed the oxidative lactonization of 250 mM 1,4-butanediol while keeping all other parameters the same (Table 1). It is worth mentioning here that for these experiments efficient supply of O_2 was essential (data not shown), which becomes clear considering the low solubility of O_2 in aqueous media (around 0.25 mM).

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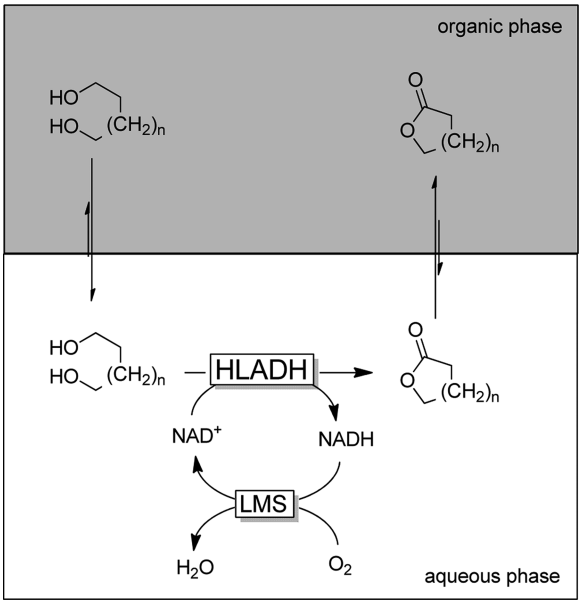
Nevertheless, also under optimized O_2-intake (after increasing the liquid−gas surface area by performing the reaction in 5-fold enlarged reaction vessels) accumulation of γ-butyrolactone (**1b**) always ceased at approximately 120 mM (48% conversion) and then gradually decreased over time, accompanied by a significant acidification of the reaction mixture to approximately pH 5 (see Supporting Information, Figure S1 for further details). The suspected hydrolysis of γ-butyrolactone could be confirmed by GC/MS analysis (see Supporting Information, Table S6). In fact, this hydrolysis was also observed with the other lactone products evaluated (Table 1). This spontaneous hydrolysis was undesired for various reasons. First, it diminished the yield of the desired lactone. Furthermore, the associated acidification of the reaction mixture led to decreased HLADH-activity (see Supporting Information, Figure S3) and impaired the stability of the nicotinamide cofactor.[11] Also, a slight product inhibition was observed for HLADH in the presence of lactones (see Supporting Information, Figure S4) requiring attention.

To circumvent these limitations, we envisioned a two-liquid-phase system (2LPS)[12] approach for the *in situ* extraction of the less hydrophilic lactone products into an organic phase, thereby circumventing or at least alleviating the lactone hydrolysis issue (Scheme 2).

Scheme 2. Using a Two-Liquid-Phase Approach to Alleviate Lactone Hydrolysis[a]



[a]The lactone formed is partially extracted into the organic layer thereby avoiding its hydrolysis. n = 1−3.

As the presence of organic solvents may impair the biocatalysts' stability,[13] we focused on solvents reported to have negligible influence on HLADH stability.[14] Though ethyl acetate exhibited good extraction properties of the lactones (see Supporting Information, Table S2), we decided against it as hydrolysis (this time of the solvent) might lead to undesired acidification. Therefore, for the proof-of-concept experiments, we used diisopropyl ether (DIPE) as organic phase. In this setup acetosyringone was substituted by 2,2′-azino-bis(3-ethylbenzthiazoline-5-sulfonic acid (ABTS). The reason was that the performance of the overall system significantly dropped under the conditions of a 2LPS setup, and full conversion could not be reached. In fact, acetosyringone significantly partitioned into the organic phase (K = 0.68, corresponding to >85% extraction into the organic phase under the reaction conditions) and thereby became unavailable for the regeneration system, whereas the dianion ABTS should not suffer from such limitations. Indeed, using ABTS as mediator (at 1 mM levels instead of 0.2 mM, to compensate for the lower activity of the ABTS-based LMS) 95% conversion of **1a** could be reached within 24 h (Figure 1), thereby doubling the

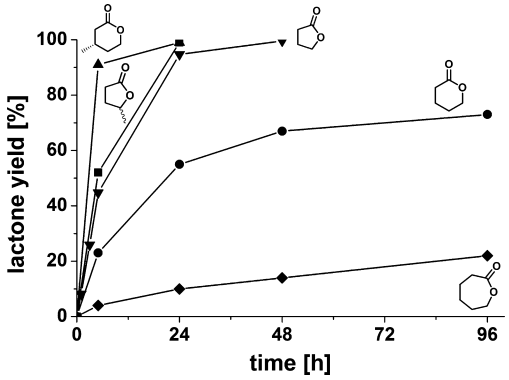


Figure 1. HLADH-catalyzed oxidation of diols to their corresponding lactones using the 2LPS approach (1b(▼), 2b(■), 3b(●), 4b(▲), 5b(♦)). Reaction conditions (aqueous, 0.2 mL): c(diol) = 250 mM, c(NAD$^+$) = 0.5 mM, c(HLADH) = c(*Mt*laccase) = 2 μM, c(ABTS) = 1 mM, organic phase: DIPE (containing 5 mM dodecane, 1.8 mL), $V_{organic}/V_{aqueous}$ = 9, buffer: Tris-HCl (50 mM, pH 8), T = 30 °C, 600 rpm. Yields are average values of duplicates.

conversion as compared to acetosyringone (see Supporting Information, Figure S5 for the comparison). As shown in Figure 1, this approach was successfully applied to the oxidative lactonization of diols **1a**−**5a**.

Gratifyingly, the enantioselectivity of the lactonization of **4a** was not impaired under these conditions. Again the optical purity of **2b** was not constant during the reaction as it decreased from 17% after 1.5 h (first sample taken) to near-racemic after 24 h (see Supporting Information, Figure S9, Table S4).

Admittedly, further improvements are necessary to turn the proposed 2LPS approach for oxidative lactonization of diols into a truly practical option for the synthesis of (optically pure) lactones. Especially, the identification of solvents for the selective extraction of the lactone products will be the focus of further investigations. As mentioned above, oxidative lactonization of diols has been known since the 1970s.[7] However, since the pioneering works by Jones et al. the substrate loadings have not been improved significantly (10−25 mM). Possibly, spontaneous lactone hydrolysis prevented higher concentrations. With the current proof of concept, we have demonstrated a promising solution to the lactone hydrolysis issue. Further improvements (especially optimized organic phases with better partitioning coefficients for the lactone products) will result in practical systems for (chiral) lactone synthesis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Further details are given in Figures S1−S10 and Tables S1−S6. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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