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Enzymatic Synthesis of Enantiopure (*R*)-Citronellal from Geraniol via a Short-Chain Dehydrogenase and Ene Reductase

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Abstract: The development of synthetic routes to produce enantiopure (*R*)-citronellal as a key intermediate for the synthesis of (–)-menthol and other valuable terpenoids is highly relevant in the pharmaceutical, flavor, and fragrance industries. Herein, we showcase a cascade with two consecutive biocatalytic steps performed separately using the inherent selectivity of a short-chain alcohol dehydrogenase (SDR) and an ene reductase (ERED) from the Old Yellow Enzyme (OYE) family. The first reaction involves the *Aa*SDR1-catalyzed oxidation of relatively inexpensive geraniol in a biphasic system, providing geranial as an intermediate. The organic phase containing geranial is then extracted and transferred to the second step, where the ERED variant OYE2_Y83V catalyzes the asymmetric reduction of geranial to produce (*R*)-citronellal, achieving >90% conversion and >99% enantiomeric excess. The use of *n*-heptane in a two-liquid phase system not only facilitates substrate and product solubilization but also minimizes geranial isomerization. This biocatalytic cascade therefore enables the synthesis of enantiopure (*R*)-citronellal.

Keywords: biocatalysis, citral, enzymatic cascades, Old Yellow Enzymes, terpenoids

1. Introduction

Citronellal is a compound with versatile use, widely appreciated for its distinct lemon, rose, and citronella scent, making it a key ingredient in flavors and fragrances.[1] Particularly, the (*R*)-enantiomer of citronellal is an essential intermediate in the industrial synthesis of (–)-menthol, a chiral compound, with significant commercial importance and one of the most widely consumed flavors globally.[1a,2] Out of eight menthol isomers, (–)-menthol possesses the particular cooling effect and desired peppermint minty odor.[1a,3] Its growing demand has driven a transition from natural extraction to chemical synthesis. Conventional synthetic pathways involve the use of *m*-cresol, citral, or myrcene, as the starting material, with the (*R*)-citronellal intermediate generally synthesized using metal catalysts with chiral ligands such as rhodium complexes (Scheme S1, Supporting Information).[4]

Although these strategies achieve high conversion, the toxic metals and complex chiral ligands employed can lead to the formation of side products and the need for extensive purification due to their imperfect selectivity (≈87% *ee* (*R*)-citronellal for the BASF process).[4] Therefore, more sustainable alternative methods should be further investigated and developed.[5]

Biocatalysis has emerged as a promising approach to address these challenges with the exquisite selectivity of

enzymes.[6] An ideal enzymatic reaction to produce enantiopure (*R*)-citronellal would be through asymmetric reduction of citral, a mixture of neral and geranial (**Figure 1**). In this sense, nicotinamide adenine dinucleotide NAD(P)H-dependent flavin mononucleotide FMN-containing ene reductases (EREDs) from the old yellow enzyme family (OYE, EC 1.6.99.1) have demonstrated their efficiency for the asymmetric reduction of a broad range of α,β-unsaturated compounds.[7] However, OYEs are currently unable to fully convert citral to enantiopure (*R*)-citronellal.[8] Screening different classes of OYEs[7a] revealed that class I converts citral to predominantly (*S*)-citronellal, class II generally reduces geranial to (*R*)-citronellal and neral to mostly (*S*)-citronellal, whereas class III typically converts both neral and geranial to enantiopure (*S*)-citronellal (Figure 1).[8b,8d]

A scale-up with OYE2.6 (class II, from *Pichia stipitis*) could achieve 67% isolated yield of (*R*)-citronellal with 98% *ee*, albeit from freshly prepared geranial.[9] Mutational studies revealed that the selectivity of NCR (class I, from *Zymomonas mobilis*) for geranial reduction could be enhanced with a double-mutant NCR W66A_I231A to 63% *ee* (*R*)-citronellal.[8b] Although promising, engineering efforts are still required for a class I OYE to produce at least >90% *ee R*. The recently characterized OYE2p (class II, from *Saccharomyces cerevisiae* YJM1341) afforded 87.2% yield and 88.8% *ee* (*R*)-citronellal from citral.[10] Upon mutagenesis, Zheng et al. obtained the single variant OYE2p_Y84V, which enabled the whole cell conversion of citral to (*R*)-citronellal with up to 95.4% *ee*.[11] Using this variant coupled with an engineered alcohol dehydrogenase (ADH), Lin and co-workers developed an in vivo whole cell cascade from 150 mM geraniol with 30% v/v hexane, affording 72.3% (*R*)-citronellal with 96.7% *ee*, still a few percent shy of enantiopurity.[12] Although the authors aimed for a redox-neutral process, the addition of 30 mM NADH was required to obtain higher conversion; therefore, further engineering of this in vivo ADH–ERED cascade is needed.

Recently, we developed an in vitro one-pot cascade using a copper radical alcohol oxidase (*Cgr*AlcOx) and OYE2 (class II, from *Saccharomyces cerevisiae* S288C) from 20 mM geraniol to (*R*)-citronellal, achieving 95.1% conversion and 95.9% *ee*.[13] One limitation was the inhibition of *Cgr*AlcOx by citronellal and isomerization of geranial to neral. To address these challenges, this study presents a biocatalytic cascade employing an ADH from the short-chain dehydrogenase (SDR) family, specifically *Aa*SDR1 from *Aedes aegypti*,[14] to catalyze the oxidation of geraniol to geranial in a two-liquid phase system, combined with OYE2 to yield enantiopure (*R*)-citronellal (Figure 1). This approach leverages the oxidation of geraniol to geranial in an aqueous-organic solvent biphasic system[15] by minimizing geranial isomerization to neral that occurs in an aqueous medium, thus enhancing the subsequent enantioselective reduction of geranial.

2. Results and Discussion

Prior to executing the full enzymatic cascade, the efficiency of each step was evaluated separately.

2.1. ADH-Catalyzed Geraniol Oxidation

To identify a suitable enzyme for selective geraniol oxidation to geranial, a screening of 17 ADHs from Johnson Matthey (C═O reduction enzyme kit EZK003) together with our purified *Aa*SDR1 (see SI) was conducted, using an NADH-oxidase (NOX) to recycle NAD(P) (**Figure 2**A). This screening revealed that ADH-19 and *Aa*SDR1 provided the most promising conversions and geranial:neral ratio after 6 h of reaction at 30 °C. As expected, increasing the *Aa*SDR1 concentration from 1 to 15 μM and reducing the reaction time to 1 h enhanced both conversion and selectivity towards geranial (Figure 2B), with 78% conversion and a 91:9 geranial:neral ratio. *Aa*SDR1's specific activity and conversions were measured in different buffers and pH units from 7.0 to 10.0, with the best overall conversion and

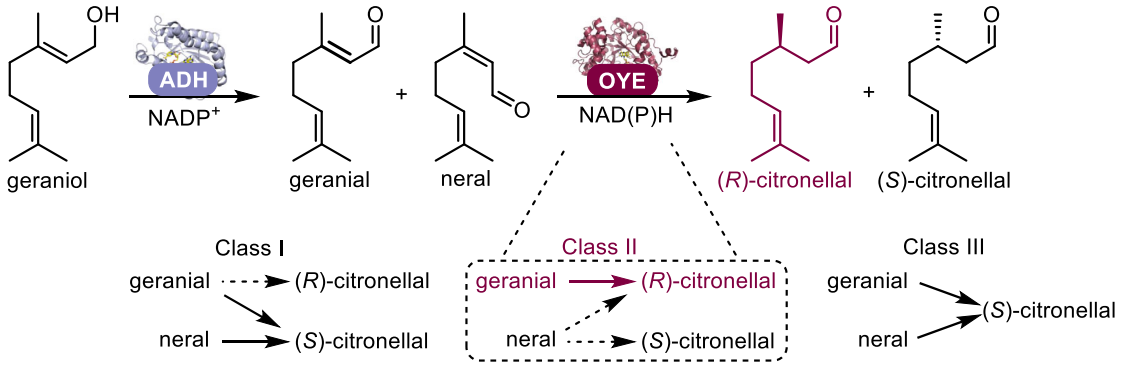

Figure 1. Enzymatic synthesis of (*R*)-citronellal from geraniol via ADH and OYE in a two-liquid phase system, including the OYE-catalyzed asymmetric reduction of citral to citronellal according to classes I–III.[7a]

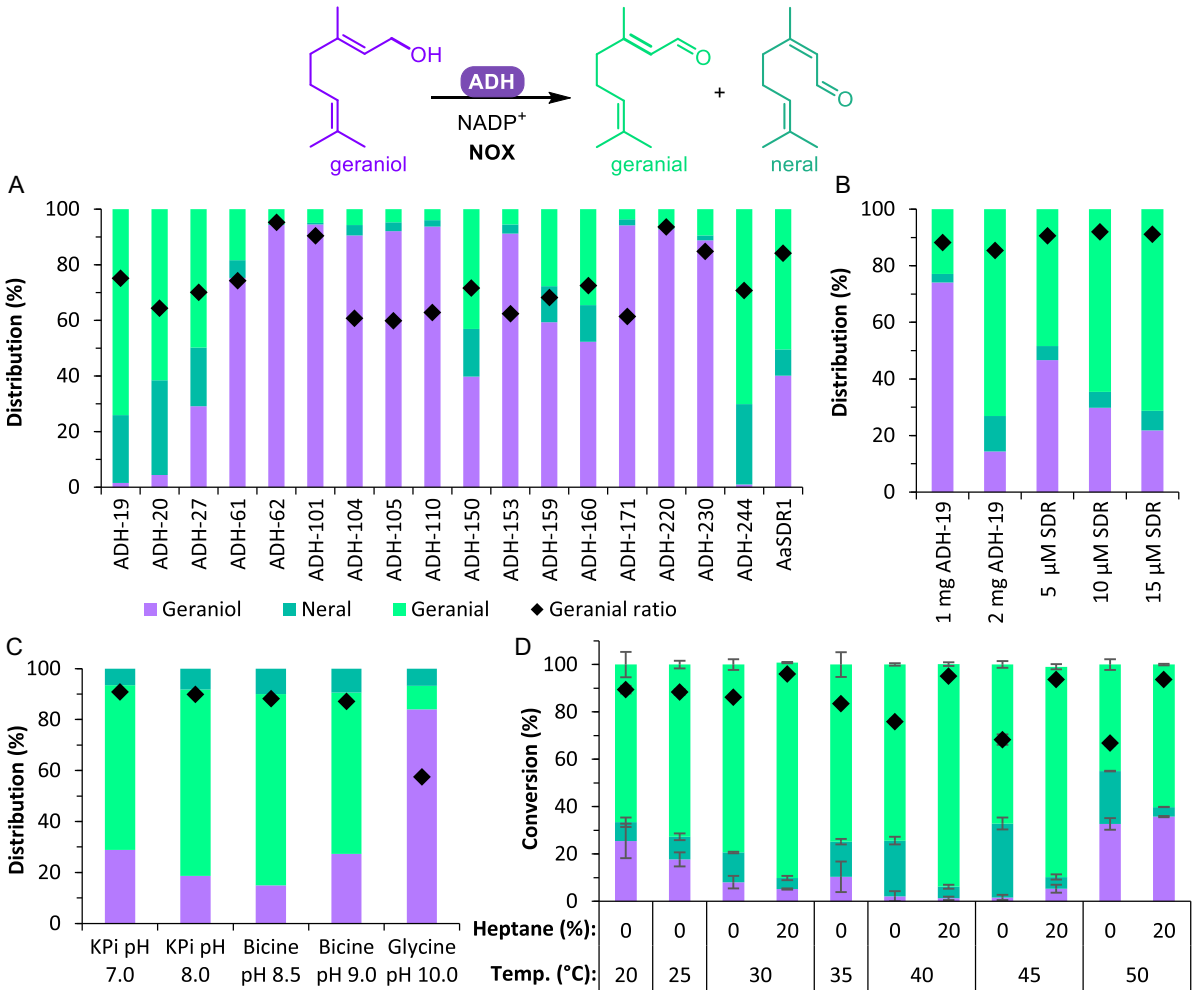

Figure 2. ADH-catalyzed geraniol oxidation. A) Screening of ADHs (CFEs from JM kit and *Aa*SDR1). Conditions: in 1 mL, 100 mM KP*i* buffer pH 8.0, 1 mM NAD(P)$^+$, 2 mg mL^{-1} NOX CFE, 1 mg mL^{-1} ADH CFE or 1 µM purified *Aa*SDR1, 10 mM geraniol (from a 1 M stock in DMSO, 1% v/v), and 30 °C, 900 rpm, 6 h. Black diamonds show geranial:neral ratio. B) ADH-19 and *Aa*SDR1 reactions in 1 h. Same conditions as (A) and 1 h. C) Influence of pH. Conditions: 50 mM buffer (MOPS-NaOH, KP*i*, Bicine, Glycine) at pH 7–10, 25 µM *Aa*SDR1, same conditions as (A) and 1 h. D) Influence of temperature and 20% v/v *n*-heptane. Conditions: in 1.2 mL, 10 mM geraniol (from a 1 M stock in DMSO, 1% v/v), 1 mM NADP$^+$, 25 µM *Aa*SDR1, 2 mg mL^{-1} NOX CFE, 0 or 20% v/v *n*-heptane, 20–50 °C, 900 rpm, and 1 h. Average of duplicates.

geranial:neral ratio with KP*i* buffer at pH 8.0 (Figure 2C, Figure S3, Supporting Information). This enzyme was stable and active (1.6–1.8 U mg^{-1}) up to pH 9.0; thereafter, conversion dropped to 26% at pH 10.0.

We also evaluated the influence of temperature and reaction medium on *Aa*SDR1-catalyzed geraniol oxidation (Figure 2D). In a buffer at 30 °C, the reaction reached 92% conversion, with 86:14 geranial:neral after 1 h. In a biphasic system containing 20% v/v *n*-heptane, conversion was 95%, with an improved 95:5 geranial:neral ratio, demonstrating the role of the organic solvent in minimizing geranial isomerization. Increasing the temperature from 30 to 40 °C with 20% v/v *n*-heptane increased the overall conversion to 99%, while preserving the 95:5 geranial:neral ratio. Conversely, in buffer, temperatures at and above 40 °C resulted in higher geranial isomerization to neral and decreased conversion. Lower temperatures at 20 and 25 °C resulted in overall lower conversions. Therefore, a temperature of 40 °C in a biphasic system with 20% v/v *n*-heptane in KP*i* buffer pH 8 was retained for further reactions. This study demonstrates, for the first time, the application and the suitability of *Aa*SDR1 in a biphasic system to maximize conversion and minimize product isomerization.

2.2. OYE-Catalyzed Citral Reduction

A screening with available OYEs from class II, OYE2, OYE3, EBP1, and *Le*OPR1 from class I was conducted to evaluate their efficiency in reducing citral to (*R*)-citronellal within 1 h (**Figure 3**A). In addition,

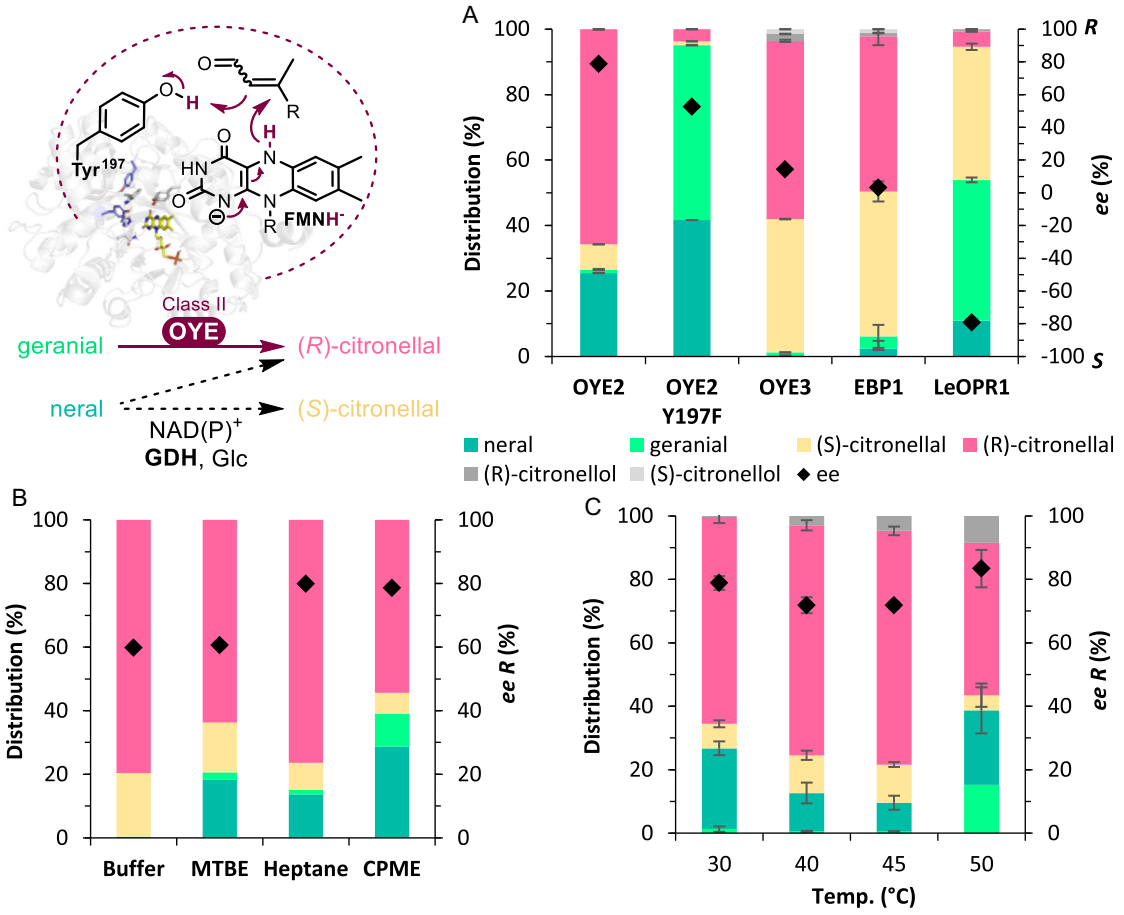

Figure 3. A) ERED screening for the asymmetric reduction of citral. Conditions: in 1 mL, 50 mM KP*i* buffer pH 8, 1 mM $NADP^+$, 20 mM glucose (Glc), 10 U mL^{-1} *Bs*GDH, 5 μM OYE, 10 mM citral (as 1% v/v in DMSO), 30 °C, 900 rpm, 1 h. Average of duplicates. B) Influence of solvents at 30 °C. Reaction in buffer: 5 μM OYE2, 4 h. Reactions with solvent: 7 μM OYE2, 20% organic solvent MTBE, *n*-heptane or CPME, 8 h. C) Influence of temperature. Conditions: same as (A), 1 h. Average of duplicates.

we tested the single mutant OYE2_Y197F, as tyrosine 197 is believed to play a key role in the active site by donating a proton to the substrate α-carbon (Figure 3).[16] OYE2 wt achieved 74% conversion from citral, with 78.8% *ee* (*R*)-citronellal (Figure 3A, values in Table S2, Supporting Information). The mutant OYE2_Y197F gave only 4% conversion, which clearly outlines the role of Y197 as a proton donor. OYE3 (from *Saccharomyces cerevisiae* S288C) and EBP1 (from *Candida albicans*) provided close to full conversion at 99% and 94%, respectively, but with lower *ee* values of 14.4% and 3.3%. *Le*OPR1 (from *Solanum lycopersicum*) showed a conversion of 57% and a clear selectivity for converting neral to the (*S*)-enantiomer, with 79.2% *ee*, which aligns with the literature.[17] Considering that the highest *ee* was obtained with OYE2, we carried out a time course profile of citral reduction in buffer, which showed that geranial is reduced to (*R*)-citronellal faster than neral, and over time neral is reduced to (*S*)- and (*R*)-citronellal (Figure S6A, Supporting Information). Full conversion was obtained in 4 h along with a significant drop to 60% *ee* (*R*)-citronellal, thus highlighting the importance of generating pure geranial from the first step of the cascade.

As previously mentioned, geranial is known to isomerize to neral in a buffer medium; therefore, a biphasic system would benefit the OYE step as well to obtain the highest (*R*)-citronellal purity.[18] Previous studies have shown that OYEs can retain activity in organic solvents, such as OYE1 (class II, from *Saccharomyces pastorianus*), which achieved conversions of 90% in up to 97% organic solvent for the asymmetric bioreduction of water labile alkene substrates.[19] Similarly, *Ts*OYE (class III, from *Thermus scotoductus*) was immobilized on celite to reduce various alkenes in methyl *tert*-butyl ether (MTBE) with minimal water.[20] OYE3 and NCR (class I, from *Zymomonas mobilis*) were also immobilized and used in up to 20% v/v organic solvent.[21] Therefore, three water-immiscible organic solvents, MTBE, *n*-heptane, and cyclopentyl methyl ether (CPME), which are usually well tolerated in enzymatic biphasic systems,[15] were evaluated and selected based on their compatibility with

the reaction system and extraction efficiency (Figure 3B). Out of the three, *n*-heptane resulted in the highest conversion of 82% in 8 h. CPME achieved a similar *ee* of 83.3%, but with an overall low conversion of 55%. A similar pattern was observed when the organic phase was increased to 30%, with lower conversions across all solvents (data not shown). Although MTBE seemed to enable better extraction capacity, 20% v/v *n*-heptane provided both the highest conversion and *ee*.

In addition, the influence of temperature on conversion was also investigated, showing higher (*R*)-citronellal formation at 45 °C (Figure 3C). However, temperatures >45 °C resulted in lower *ee* values due to increased geranial isomerization to neral. Based on these findings, 40 °C was selected for subsequent experiments.

2.3. Full Cascade: *Aa*SDR1-OYE2-Catalyzed Production of (*R*)-Citronellal from Geraniol

The initial approach for producing (*R*)-citronellal from geraniol involved a one-pot redox neutral bienzymatic cascade in a fully aqueous medium. This system consisted of two main steps: first, the oxidation of geraniol to geranial by *Aa*SDR1, with $NADP^+$ as the hydride acceptor. Subsequently, geranial was reduced to (*R*)-citronellal catalyzed by OYE2, using the NADPH generated in the first step, thereby recycling the cofactor. However, during the combined enzymatic reactions, *Aa*SDR1 not only oxidized geraniol to citral but also reduced (*R*)-citronellal to (*R*)-citronellol. The side reaction resulted in 85.2% of the (*R*)-citronellal converted to (*R*)-citronellol, thereby significantly reducing the yield toward the desired (*R*)-citronellal product. This over-reduction presented a challenge to the one-pot redox-neutral cascade, requiring the development of an alternative approach. Consequently, a biphasic enzymatic system was developed, involving the two consecutive separated steps to overcome this issue and enhance the overall efficiency and specificity of (*R*)-citronellal production.

Taking the ideal conditions determined in the first step, *Aa*SDR1-catalyzed geraniol oxidation was carried out at 40 °C with 20% v/v *n*-heptane for 1 h, after which the geranial was extracted with *n*-heptane and introduced directly to the second step of the cascade. The OYE2-catalyzed geranial reduction was thus carried out in the biphasic system at 40 °C. A time-course study of conversion and *ee* values was conducted over a period of 4 h (**Figure 4**, values in Table S3 entries 1–4, Supporting Information). As anticipated, conversion increased over time, though a slight decrease in *ee* was observed from 95% to 94%, ascribed to the presence of trace amounts of neral converted to (*S*)-citronellal.

Despite these improvements, 6–8% (*R*)-citronellol emerged as a side product, attributed to the promiscuous activity of *Bs*GDH, which reduces (*R*)-citronellal to (*R*)-citronellol. To mitigate this, we experimented with

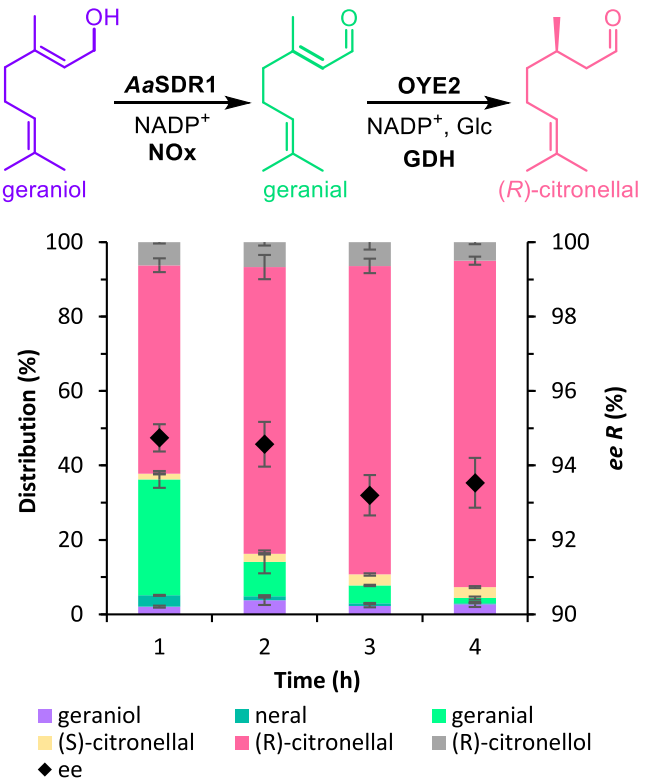

Figure 4. *Aa*SDR1-OYE2 enzymatic cascade from geraniol to (*R*)-citronellal. Conditions: **1st step:** 50 mM KP*i* pH 8, 2 mg mL^{-1} CFE NOX, 1 mM $NADP^+$, 25 µM *Aa*SDR1, 10 mM geraniol (1% v/v DMSO), 200 µL *n*-heptane, 1.2 mL final volume, 40 °C, 900 rpm, 1 h. **2nd step:** 50 mM KP*i* pH 8, 50 mmol Glc, 1 mmol $NADP^+$, 10 U mL^{-1} *Bs*GDH, 10 µmol OYE2 wt, and 200 µL *n*-heptane mixture containing geranial from the first step, 1 mL final volume, 40 °C, 900 rpm. Average of duplicates.

varying amounts of *Bs*GDH in the cofactor recycling system (**Table 1**, entries 1–4). Decreasing the amount of *Bs*GDH from 10 to 7 or 5 U mL^{-1} decreased (*R*)-citronellol production to 4%. An alternative recycling system using formate or phosphite dehydrogenase could be used to further prevent this over-reduction to citronellol.

To enhance the selectivity of the second step, we produced the single OYE2 mutants Y83V, T38A, and T38C (Table S2, Supporting Information). OYE2_Y83V afforded the best overall performance with 76% conversion and 94.6% *ee*. The T38A and T38C mutants exhibited lower conversions at 60% and 36% respectively, yet similar *ee* values compared with OYE2 wt (Table S2, entries 3–4, Supporting Information).

Overall, the OYE2_Y83V mutant distinguished itself by its higher conversion and improved enantioselectivity, which aligns with the results of Zheng et al. with the OYE2p_Y84V mutant (equivalent position to OYE2_Y83V).[10,11] From molecular docking and dynamics simulations, Wei and co-workers showed that this mutation provides shorter distances for hydride and

Table 1. *Aa*SDR1-OYE2 enzymatic cascade for the production of (*R*)-citronellal at 40 °C.[a)]

Entry	OYE2	GDH [U mL^{-1}]	Time [h]	Total conv. [%]	(*R*)-citronellal [%]	*ee* [%]
1	wt	5	3	88.3 ± 1.1	82.4 ± 1.0	94.2 ± 0.1
2	wt	10	4	93.9 ± 0.9	84.7 ± 1.2	94.9 ± 0.0
3	wt	7	4	93.4 ± 0.4	86.1 ± 0.3	94.6 ± 0.1
4	wt	5	4	91.6 ± 0.9	85.1 ± 1.0	94.3 ± 0.1
5	Y83V	10	4	88.3 ± 1.7	80.3 ± 1.9	99.2 ± 0.0
6	Y83V	5	4	91.8 ± 2.2	88.6 ± 2.1	99.0 ± 0.0

[a)] See conditions in Figure 4; geraniol purity 98%. Total conversion = geraniol consumption. Average of duplicates.

Table 2. OYE2-specific activities obtained with citral and cyclohexenone.

Substrate	OYE2	Spec. act. [U mg^{-1}][a)]
Citral	wt	0.37 ± 0.01
Citral	Y83V	1.38 ± 0.05
Cyclohexenone	wt	3.63 ± 0.08
Cyclohexenone	Y83V	3.23 ± 0.02

[a)] Conditions: 50 mM KP*i* buffer pH 8, 0.2 mM NADPH, 10 mM substrate from a 1 M stock in DMSO), OYE, at 40 °C for citral and at 25 °C for cyclohexenone. Average of duplicates.

proton transfer to both geranial and neral, improving its catalytic efficiency.[11] Moreover, the mutation induces a modified (*R*)-selective binding mode for neral, increasing the selectivity. The specific activity of OYE2 wt and Y83V was measured for both citral and cyclohexenone as a benchmark substrate (**Table 2**). The mutant Y83V displayed an almost fourfold increase in activity for citral reduction, from 0.37 to 1.38 U mg^{-1}, confirming the higher conversion observed, whereas the activity for cyclohexenone reduction remained similar at 3.23–3.63 U mg^{-1}.

Subsequent full cascade experiments with the mutant OYE2_Y83V showcased the highest selectivity for producing (*R*)-citronellal, reaching 88–92% conversion and 99.2–99.0% *ee*, with 3.9–7.6% geraniol remaining and 2.8–7.7% (*R*)-citronellol with 10 and 5 U mL^{-1} *Bs*GDH, respectively (Table 1, entries 5–6). A scale-up of 18 mg led to 88.5% overall conversion, yielding 77.5% (*R*)-citronellal with an *ee* of 99.4% (see SI for details). This represents the highest *ee* reported for (*R*)-citronellal. The performance of OYE2_Y83V highlights its potential as an effective biocatalyst, setting a new standard for enantioselectivity in the biosynthesis of (*R*)-citronellal. Further implementation of this variant for other cascades and scale-up is ongoing.

3. Conclusion

In this study, we developed an enzymatic cascade with an SDR and OYE2 that produces enantiopure (*R*)-citronellal from cost-effective and available geraniol. The design of this approach in a 20% v/v *n*-heptane-buffer biphasic system effectively minimizes the isomerization associated with the reduction of citral by OYEs. The OYE2_Y83V variant significantly enhanced selectivity, achieving an unprecedented 99.4% *ee*, the highest value reported for (*R*)-citronellal production. This advancement underscores the potential of this methodology as a synthetic route to enantiopure (*R*)-citronellal, which can directly serve as a key intermediate in the synthesis of (–)-menthol and other valuable terpenoids. The suitable combination of *Aa*SDR1 and the OYE2_Y83V mutant could be enhanced to become a redox-neutral process, via engineering of the SDR toward higher oxidative activity, or via immobilization[22] and in a flow system, which has been gaining a lot of interest.[23]

4. Experimental Section

General Remarks: Chemicals were obtained from Merck Sigma-Aldrich, TCI Europe, abcr GmbH and used as received. In particular, the following chemicals were obtained from Merk Sigma-Aldrich, geraniol (both as >98% and 90% purity, CAS 106-24-1), citral (>95%, CAS 5392-40-5), (*R*)-(+)-citronellal (CAS 2385-77-5), and (*S*)-(–)-citronellal (CAS 5949-05-3), and NADPH (CAS 2646-71-1) was purchased from the Oriental Yeast Co. (OYC Europe), and NADP$^+$ (97.6%, CAS 698999-85-8) from Prozomix Ltd (UK).

Enzymes OYE2 wt/T38C/T38A/Y83V/Y197F, OYE3, EBP1, *Le*OPR1,[24] *Aa*SDR1, *Bs*GDH_E170K_Q252L, and *Sm*NOX2_V193R_V194H were recombinantly produced in *E. coli* and purified by affinity chromatography as detailed in the SI. The JM C=O reduction enzyme kit EZK003 was gratefully received from Johnson Matthey (Cambridge, UK). Commercial NAD(P)H oxidase (NOx, CAS 77106-92-4) was purchased from Prozomix Ltd (UK).

Bioconversions: Biocatalytic reactions were carried out in a final volume of 1 or 1.2 mL in safe-lock Eppendorf 2 mL plastic tubes. The reaction mixtures were shaken at 900 rpm in an Eppendorf ThermoMixer C with a ThermoTop, at specified temperatures and times, and incubated on ice for 2 min before extraction.

ADH-Catalyzed Geraniol Oxidation: Conditions for the JM ADH CFEs and *Aa*SDR1 screening for geraniol oxidation were: in 1 mL, 100 mM KP*i* buffer pH 8.0, 1 mM NAD(P)$^+$, 2 mg mL^{-1} NOX, 1 mg ADH or 1 µM *Aa*SDR1, and 10 mM geraniol (from a 1 M stock in DMSO, 1% v/v), at 30 °C, 6 h, extracted with 500 µL

EtOAc containing 5 mM dodecane as internal standard, vortexed, and centrifuged (14 000 rpm, 7 min). The organic layer was separated, dried over anhydrous $MgSO_4$, and transferred to a GC vial for analyses. Samples containing an additional 20% v/v *n*-heptane were subjected to a two-step extraction process. The first extraction was performed using *n*-heptane, followed by a second extraction with an additional 125 µL of *n*-heptane.

ERED-Catalyzed Citral Reduction: ERED-catalyzed reduction of citral was carried out in 1 mL, 50 mM KP*i* buffer pH 8, 20 mM Glc, 1 mM $NADP^+$, 10 U mL^{-1} *Bs*GDH, 5 µM ERED, and 10 mM citral (from a 1 M stock in DMSO, 1% v/v), at 30 °C, 1 h. For the biphasic system, 200 µL *n*-heptane were added. Reaction products were extracted with EtOAc containing 5 mM dodecane, vortexed, and centrifuged (3 min, 14 000 rpm). The organic layer was separated, dried over anhydrous $MgSO_4$, and transferred to a GC vial for analysis.

Full Cascade Reactions: Conditions: **1st step:** 50 mM KP*i* pH 8, 10 mM geraniol (1% v/v DMSO), 2 mg mL^{-1} CFE NOX, 1 mM $NADP^+$, 25 µM *Aa*SDR1, and 200 µL *n*-heptane, 1.2 mL final volume, 40 °C, 900 rpm, 1 h. **2nd step:** 50 mM KP*i* pH 8, 50 mmol Glc, 1 mmol $NADP^+$, 10 U mL^{-1} *Bs*GDH, 10 µmol OYE2 wt, and 200 µL *n*-heptane mixture containing geranial from the first step, 1 mL final volume, 40 °C, 900 rpm. Following incubation on ice, the reaction products were extracted with *n*-heptane, dried over anhydrous $MgSO_4$, and transferred to a GC vial for analysis.

Analytical Procedures: Gas chromatography was performed on Shimadzu GC-2010 gas chromatographs (Shimadzu corporation, Kyoto, Japan) equipped with a flame ionization detector and the chiral column Hydrodex ß-TBDAc (Macherey-Nagel, Germany, 50 m × 0.25 mm × 0.25 µm). Products were confirmed by reference standards and GC–MS. Product concentrations were obtained with calibration curve equations using an internal standard in the EtOAc used for extraction.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

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