

Selective Enzymatic C–H-Oxyfunctionalization for the Efficient Synthesis of Grevillic Acid

Clemens Cziegler,^a Benjamin Baumert,^a Christoffel P. S. Badenhorst,^a Karsten Siems,^b and Uwe T. Bornscheuer^{a,*}

^a Dept. of Biotechnology and Enzyme Catalysis, Institute of Biochemistry, University of Greifswald, Felix-Hausdorff-Str. 4, 17487 Greifswald, Germany

E-mail: uwe.bornscheuer@uni-greifswald.de

^b AnalytiCon Discovery GmbH, Herrmanswerder 17, 14473 Potsdam, Germany

Manuscript received: November 17, 2024; Revised manuscript received: November 27, 2024;

Version of record online: December 4, 2024



Supporting information for this article is available on the WWW under <https://doi.org/10.1002/adsc.202401421>

© 2024 The Author(s). Advanced Synthesis & Catalysis published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

Abstract: In this study, a selective C–H-oxyfunctionalization using an unspecific peroxygenase (UPO) enabled an efficient biosynthetic route for the synthesis of grevillic acid (GA), a natural antioxidant. The route commenced with the release of *o*-coumaric acid (oCA) from *trans*-2-coumaric acid glucoside (*trans*-CAG) using commercially available β -glucosidase from almonds. In addition, a *cis*-to-*trans* photoisomerization of *cis*-CAG was implemented to increase the synthetic access of oCA. The key step, the *para*-hydroxylation relative to the existing hydroxyl group of oCA, was accomplished by an UPO with full conversion and 93% isolated yield. Despite its name, the UPO turned out to exhibit excellent regioselectivity in this C–H functionalization, requiring only H₂O₂ as a cosubstrate.

Keywords: Antioxidants; Biocatalysis; Hydroxylation; Photoisomerization; Unspecific peroxygenase

Selective C–H-oxyfunctionalization of aromatics remains one of the most pivotal challenges in synthetic chemistry.^[1–4] Traditional chemical methods often lack efficiency and selectivity and produce over-oxygenated by-products.^[3] Furthermore, these reactions typically require harsh reaction conditions such as high temperature and pressure, and they frequently utilize organic solvents and inorganic catalysts, making them environmentally unfriendly.^[5,6] Phenols, however, are valuable

synthons in the production of dyes, agrochemicals, pharmaceuticals, and polymers, while polyphenols are renowned for their potent antioxidative properties, finding applications in many consumer products, including processed foods and cosmetics.^[7,8] Consequently, the manufacture of (poly)phenolic compounds under benign reaction conditions is highly demanded.

Enzymes, evolved by nature to catalyze (complex) transformations with high regio-, stereo-, and chemoselectivity, offer promising synthetic tools for developing more sustainable processes for the production of phenolic compounds.^[9] In this context, several enzyme classes have been studied to catalyze the hydroxylation of aromatics. For example, Li et al. reported the selective *para*-hydroxylation of *meta*-alkylphenols using mutants of the cytochrome P450 enzyme BM3.^[10] Similarly, flavin-dependent monooxygenases (FMOs) have also been found to catalyze the selective *ortho*- or *para*-hydroxylation of phenols. 3-Hydroxybenzoate 4-hydroxylase (MHBH) from *Comamonas testosteroni* KH122-3 s and 3-hydroxybenzoate 6-hydroxylase (3HB6H) from *Rhodococcus jostii* RHA1 have been reported to catalyze the conversions of 3-hydroxybenzoate to 3,4-dihydroxybenzoate and 2,5-dihydroxybenzoate, respectively.^[11,12] Another interesting example are the 4-hydroxyphenylacetic acid 1-hydroxylases (4-HPAH-1) from *Delftia acidovorans*^[13] and *Halomonas titanicae*.^[14] This two-component enzyme system consists of a flavin-dependent oxidase and a mutase, which catalyze the conversion of 4-hydroxyphenylacetic acid (4-HPA) to homogentisic acid (HGA). In the last few years, unspecific peroxygenases (UPOs)

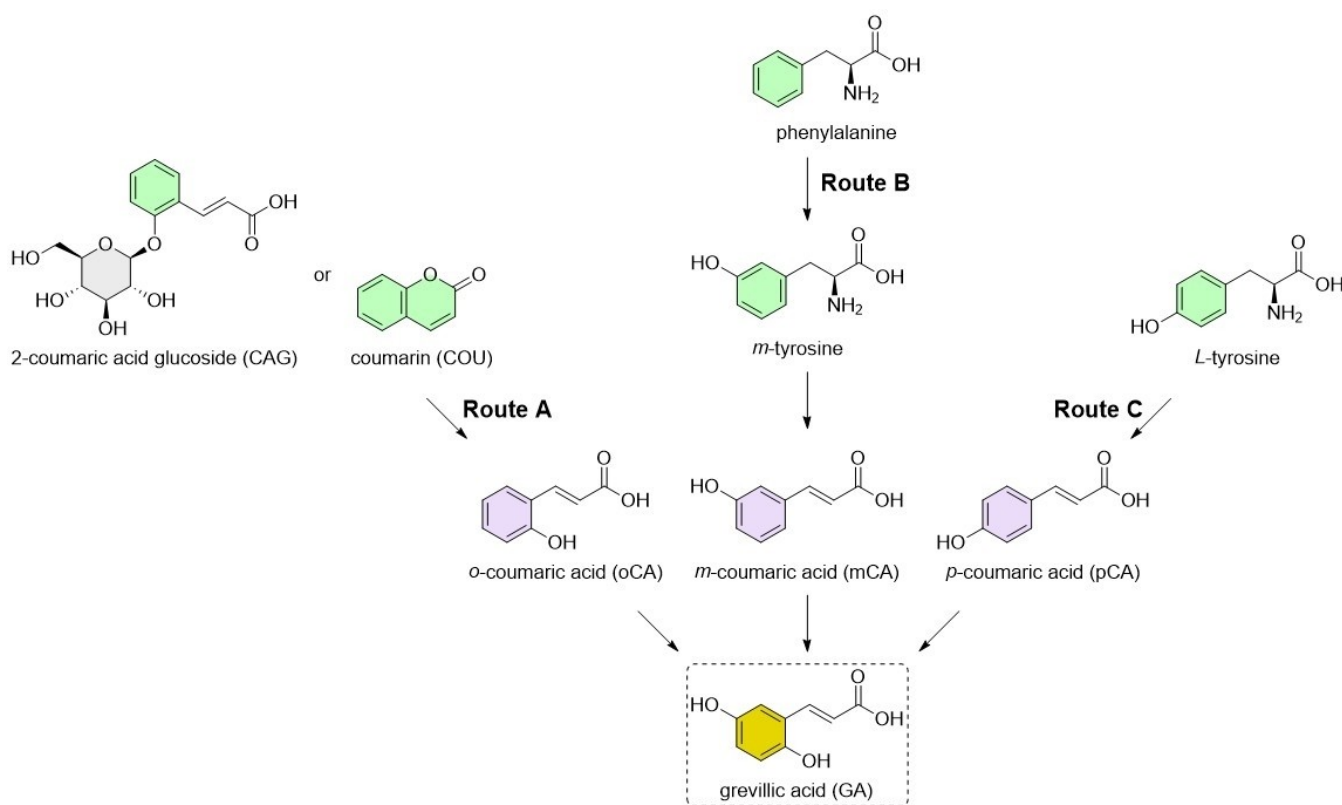
have gained significant interest in the field of organic synthesis.^[15–17] In contrast to P450s and FMOs, they do not rely on expensive cofactors such as NAD(P)H or flavins, requiring only H₂O₂ as an oxidant to fulfill their catalytic cycle.^[16] UPOs have been investigated for aliphatic hydroxylation^[18–20] and for aromatic ring hydroxylation. The first discovered UPO from *Agrocybe aegerita*^[21] has been shown to regioselectively hydroxylate benzene, phenol,^[22] flavonoids,^[23] and naphthalenes.^[24] Furthermore Schmitz et al. recently described the selective hydroxylation of arenes and phenols using an UPO from *Aspergillus brasiliensis*.^[25]

Grevillic acid (GA) is a phenolic compound belonging to the class of hydroxycinnamic acids (HCAs) with potent antioxidative properties,^[26,27] making GA useful for applications in the food, cosmetic, and pharmaceutical industries. GA or its methyl ester can be found in *Grevillea robusta*,^[28] *Murraya paniculata*,^[29] and *Heliciopsis lobata*.^[30] However, similar to other natural compounds, its isolation is inefficient, time-consuming, and costly.^[31,32] Currently, the biosynthetic pathway of GA remains unknown, and its synthesis poses significant challenges due to its

complex structure and the need for precise control over the hydroxylation sites. Therefore, we set out to develop a *de novo* biosynthetic route using readily available natural substrates to meet global trends toward more sustainable production of natural compounds.

We commenced our investigations by identifying possible biosynthetic routes toward GA (Scheme 1). GA is a dihydroxycinnamic acid with the two hydroxyl groups at the 2- and 5-positions of the phenyl ring.

To access this challenging substitution pattern, we envisioned *o*-coumaric acid (oCA) and *m*-coumaric acid (mCA) as potential substrates, where *para*-hydroxylation relative to the existing hydroxyl group would provide access to GA (Scheme 1, Routes A and B). Additionally, we considered *p*-coumaric acid (pCA) as an alternative substrate, following Hareland et al.'s report that 4-hydroxyphenyl propionic acid, which only lacks the double bond of GA, serves as a substrate for 4-HPAH-1. Thus, *para*-hydroxylation of pCA relative to the existing hydroxyl group and concomitant alkyl shift via 4-HPAH-1 would form GA (Scheme 1, Route C). oCA could be synthesized by the



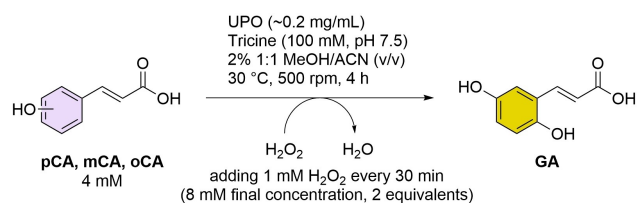
Scheme 1. Possible biosynthetic routes toward the synthesis of grevilliac acid (GA). In Route A, *o*-coumaric acid (oCA) could be formed via the deglycosylation of 2-coumaric acid glucoside (CAG) or hydrolysis of coumarin (COU). In Route B, *m*-coumaric acid could be synthesized starting from phenylalanine, which is first converted to *m*-tyrosine using a phenyl 3-hydroxylase (Phe3H).^[33,34] Deamination of *m*-tyrosine using a phenylalanine lyase would give mCA.^[35] In Route C, *p*-coumaric acid (pCA) could be synthesized from *L*-tyrosine by a tyrosine ammonia lyase.^[36] In all routes, *para*-hydroxylation relative to the existing hydroxyl group (and concomitant alkyl shift in the case of pCA) results in the formation of GA.

enzymatic hydrolysis of coumarin (COU), found in tonca beans.^[37] Alternatively, the deglycosylation of 2-coumaric acid glucoside (CAG), found in *Melilotus albus*,^[38] would release oCA. The synthesis of mCA could involve the hydroxylation of L-phenylalanine at the C-3 position, as demonstrated by Zhang et al.^[33] and Grünschow et al.^[34] using a phenyl 3-hydroxylase (Phe3H) from *Streptomyces coeruleorubidus*. The required deamination of *m*-tyrosine to afford mCA was reported by Lovelock et al.^[35] using a phenylalanine ammonia lyase (PAL) (Scheme 1, Route B). pCA could be synthesized from L-tyrosine by a tyrosine ammonia lyase (TAL).^[36] The *para*-hydroxylation relative to the existing hydroxyl group in all routes is particularly challenging due to the necessity of regioselective hydroxylation and the possibility of over-oxidation. Chemical methods are limited by poor selectivity and unsustainability. Enzymatic hydroxylation, however, presents a promising alternative but requires suitable enzymes achieving high selectivity. Our study addresses these challenges with the aim to establish a robust, efficient, and eco-friendly method for synthesizing GA.

We initiated our study of finding a suitable enzyme for the synthesis of GA by investigating the 4-HPAH-1 enzyme system using pCA as substrate. We prepared cell-free lysates from *Delftia acidovorans* and *Halomonas titanicae*, cultivated in minimal media with 4-HPA as the sole carbon source to trigger the expression of the 4-HPAH-1 enzyme system,^[13,14] and employed 4-HPA, 4-hydroxyphenyl propionic acid, and pCA as substrates. However, only the conversion of HPA to HGA could be confirmed via TLC analysis, indicating that 4-HPAH-1 has no enzymatic activity toward pCA.

Consequently, we turned our attention to the screening of 44 UPOs (UPO enzyme panel, Aminoverse B.V.) for their potential to catalyze *para*-hydroxylation of pCA, mCA, or oCA (Scheme 2).

In a first approach, we used $\sim 0.2 \text{ mg mL}^{-1}$ lyophilized enzyme, 4 mM substrate, and 1 mM H_2O_2 , added every 30 min for 4 h, in 100 mM tricine buffer (pH = 7.5) at 30 °C. Biotransformations with pCA did not show any GA formation according to TLC analysis.



Scheme 2. Screening of 44 unspecific peroxygenases (UPOs). Conditions: substrate (4 mM; pCA, mCA, or oCA), lyophilized UPO (0.2 mg mL^{-1}), H_2O_2 (1 mM added every 30 min), 100 mM tricine buffer (pH 7.5), MeOH/ACN 1:1 2% (v/v), 4 h, temperature 30 °C.

Most of the reactions using mCA and oCA as substrates also did not result in detectable GA formation. However, potential hits were identified when mCA or oCA were employed as substrates. Reactions with mCA also revealed the formation of caffeic acid, whereas only GA was detected when oCA was used. These preliminary results were further validated through gas chromatography-mass spectrometry (GC-MS) analysis compared against commercially available standards of GA and caffeic acid (Figures S4–S6). Based on these initial findings, we selected two UPOs (UPO2 and UPO23) to further assess their potential for GA synthesis using oCA. This decision was built on the observation that the hydroxylation of oCA appears more selective than that of mCA and also offers more efficient synthetic access since oCA could be obtained in a single step either from CAG or COU, whereas mCA requires at least two steps (Scheme 1).

Before further studying the synthesis of GA, we first investigated the operational robustness of UPO2 and UPO23 by assessing their solvent and temperature tolerance (Figure 1). ABTS was chosen as substrate, allowing the use of a convenient spectrophotometric assay for activity measurement.^[39] Investigations on the tolerance of common organic solvents, including dimethyl sulfoxide (DMSO), acetone, methanol (MeOH), and acetonitrile (ACN), revealed a pronounced sensitivity of both enzymes to DMSO. This observation aligns with previous studies that reported DMSO's inhibitory effects on UPOs, potentially due to its interaction with the iron center of these enzymes, thus hindering substrate access to the active site.^[39,40] Conversely, the presence of ACN was found to enhance the activity of UPO2 toward ABTS, while the activity of UPO23 decreased by approximately 50% with 2% (v/v) ACN. Acetone had a relatively mild effect on both enzymes, suggesting that concentrations between 5–10% (v/v) can be used without significantly impacting enzymatic activity. UPO23 showed good tolerance toward MeOH, whereas the activity of UPO2 was reduced by half at 2% MeOH. We further studied the temperature tolerance of both enzymes by measuring the activity toward ABTS after incubation at various temperatures and times (Figure 1A and B). UPO2 displayed good stability at 30 °C and 40 °C. When UPO2 was incubated at 50 °C, it lost 40% of its activity after 30 min and about 60% after 90 min. A rapid inactivation of UPO2 was noticed at 60 °C, losing all its activity within 60 min of incubation. In contrast, UPO23 was mostly inactivated within 10 to 20 min at all investigated temperatures, indicating that UPO23 has limited operational robustness. Consequently, we selected UPO2 and shifted our focus to evaluate the optimal conditions for the synthesis of GA.

Initially, we observed only inefficient formation of GA, yielding 0.31 mM from 4 mM oCA after 2 hours when 1 mM H_2O_2 was added every 15 min (Figure 2).

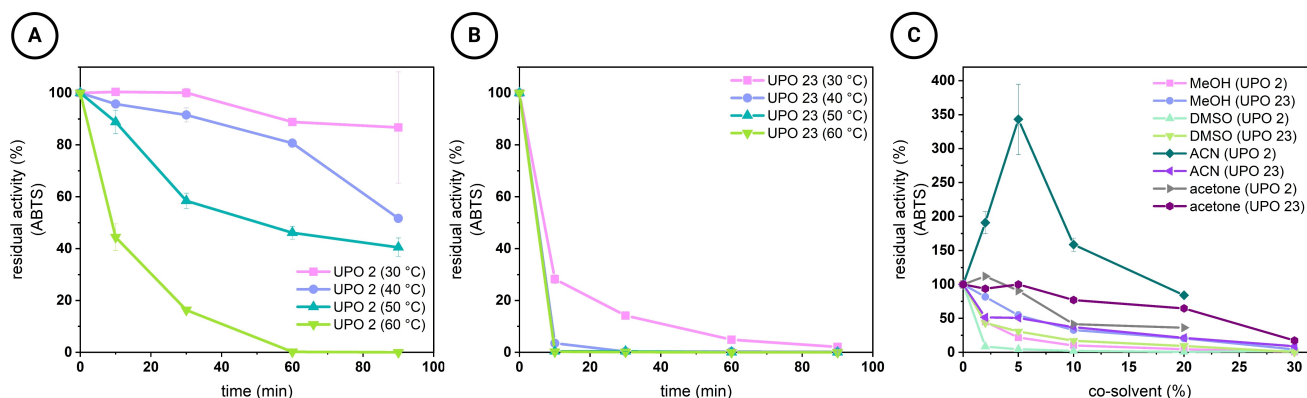


Figure 1. Biochemical characterization of UPO2 and UPO23. (A) and (B) Thermal inactivation was evaluated using 100 mM sodium citrate (pH 4.5), ABTS (30 μ M), H₂O₂ (2 mM), enzyme solution (appropriately diluted to ensure a linear response in kinetic mode). (C) Co-solvent tolerance was assessed in a reaction mixture consisting of 100 mM sodium citrate (pH 4.5), ABTS (30 μ M), H₂O₂ (2 mM), enzyme solution (appropriately diluted to ensure a linear response in kinetic mode). Co-solvent concentration is indicated as % (v/v). The conversion of ABTS was monitored by measuring the absorbance at 420 nm ($\epsilon_{420} = 36,000 \text{ M}^{-1} \text{ cm}^{-1}$) for a duration of 120 s at 25 °C. Reactions were performed as triplicate ($n = 3$).

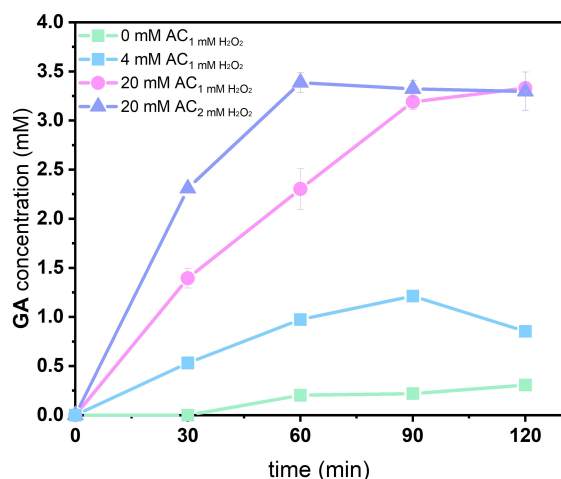


Figure 2. Time-course study of the transformation of oCA to GA. Conditions: oCA (4 mM), lyophilized UPO2 (0.2 mg mL⁻¹), Ascorbic acid (AC, 0, 4, or 20 mM), H₂O₂ (1 or 2 mM added every 15 min), 100 mM tricine (pH 7.5), MeOH/ACN 1:1 2% (v/v), 500 rpm, 2 h, temperature 30 °C. Samples were taken after indicated time points, derivatized using BSTFA/pyridine, and stored at 4 °C in the dark before analysis via GC-MS. Reactions were performed in duplicate ($n = 2$).

We reasoned that the peroxidase activity of UPO2 might cause the limited GA formation due to phenoxy radical formation and subsequent polymerization.^[17,41] Hence, we added 4 mM ascorbic acid (AC) as a radical scavenger to the biotransformation and found an increase in GA to 1.21 mM after 1.5 hours. However, we detected a lower formation of 0.85 mM GA after 2 hours, suggesting that the scavenging effect was insufficient. Increasing the concentration of AC to 20 mM resulted in 3.33 mM GA from 4 mM oCA within

2 hours. Despite its name, UPO2 transformed oCA to GA with remarkable regio- and chemoselectivity, as only the *para*-hydroxylated product and no multi-hydroxylated compounds were observed. Similarly, a preferred *para*-hydroxylation was already reported for the hydroxylation of phenol to hydroquinone.^[22] Additionally, no epoxidation of the double bond was observed.

Next, we evaluated the optimal H₂O₂ concentration. Adding 2 mM H₂O₂ instead of 1 mM every 15 min positively influenced the catalytic activity, achieving 3.39 mM GA within one hour. However, controlling H₂O₂ concentration is crucial to prevent polymerization reactions and enzyme degradation, as further H₂O₂ addition resulted in a slight decrease in GA. Then, we set out to determine the optimal temperature and pH for converting oCA to GA. We observed that the temperature did not significantly affect GA formation (Figure S1), whereas lower pH values generally favored the formation of GA (Figure S2). However, tricine buffer at pH 7.5 yielded similar good results. Of the investigated buffers, we selected 100 mM sodium acetate (NaOAc, pH 4.0) as the buffer of choice. Although Na-citrate buffer (pH 3) facilitated good GA formation in the pH study (Figure S2), citric acid's solubility in the extraction solvent ethyl acetate (EtOAc) would require an additional purification step, such as column chromatography. Furthermore, higher pH values (such as those with tricine buffer) promote the oxidation of phenols,^[42,43] as demonstrated in the time-course study with NaOAc (pH 4), which showed a higher formation of GA (Figure S3) compared to tricine buffer (Figure 2), especially in the absence of the antioxidant.

Under optimal conditions, we could decrease the enzyme concentration from initially 0.2 mg mL⁻¹ to

0.05 mg mL⁻¹. Then, we conducted a preparative scale reaction (50 mg oCA, 3.5 mg UPO2 lyophilized cell lysate). The reaction progress was monitored by TLC analysis, and upon completion, 53 mg GA (93% isolated yield) was obtained after a simple extractive workup.

With the synthesis of GA established, we shifted our attention to the biosynthesis of oCA. In a first approach, we investigated the hydrolysis of COU. Therefore, we compiled a panel of hydrolases (Table S1) comprising eight commercially available lipases, two esterases, and three lactonases. The experimental procedures, SDS-PAGE of the expressed lactonases (Figure S7), and the figures of the enzymatic activities (Figure S8 and S9) can be seen in the Supporting Information (SI). Despite the diverse enzymatic collection, we did not observe any oCA formation, possibly due to the formation of the *cis*-isomer of oCA, which readily recycles upon acidification. Hence, the alternative route, the deglycosylation of CAG, was studied. The biosynthetic route toward GA starting from CAG is shown in Figure 3A (see also Scheme 1). CAG exists in the *cis*- and *trans*-configurations and can be isolated from *Melilotus albus*. We employed commercially available β -glucosidase from almonds, leveraging the optimal conditions previously established by our group.^[44] First, the deglycosylation of the *trans*-isomer of CAG was investigated using 0.2 mg mL⁻¹ enzyme and 1 mM CAG in a 100 mM NaOAc buffer (pH = 5.5). The reaction was incubated at 35 °C and, after 20–24 h, 1 mM oCA was obtained (Figure 3B). Under the same conditions, a preparative scale reaction (30 mg *trans*-CAG) was performed, obtaining oCA in quantitative yield after extractive workup.

When the *cis*-isomer of CAG was employed, we only observed the formation of COU, indicating the cyclization to form the lactone after the release of the sugar moiety.

This lactonization can also be observed in the biosynthesis of coumarin, where *cis*-CAG is a direct precursor.^[45] This may explain why *cis*-CAG is found as the major isomer in *Melilotus albus*, as coumarin is potentially significant for the plant's defense mechanisms.^[46] Consequently, to further improve the outcome of our synthetic route, we opted for *cis*-to-*trans* isomerization of *cis*-CAG. First, no *trans*-CAG was observed when a chemical isomerization^[47] method was applied to *cis*-CAG. Hence, the focus was then placed on photoisomerization.^[48] When the *cis*-CAG was irradiated without the addition of a photocatalyst, no formation of *trans*-CAG was detected. Therefore, it was decided to add photocatalysts with different triplet-state energies (E_T).

While no formation of *trans*-CAG was detected in the presence of riboflavin ($E_T = 209$ kJ mol⁻¹),^[49] the addition of the photocatalysts benzophenone ($E_T =$

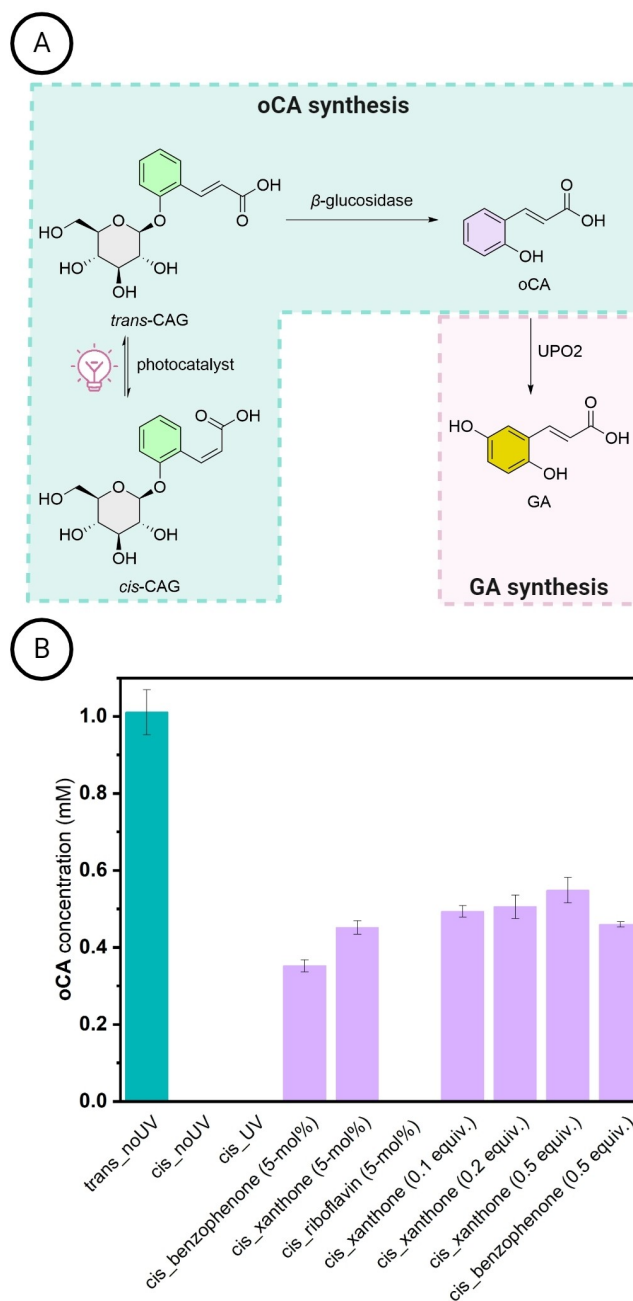


Figure 3. Biosynthetic pathway for the synthesis of oCA including photoisomerization and deglycosylation of CAG in addition to the UPO-catalyzed oxidation to GA. (A) Scheme of the biosynthetic route for the synthesis of GA. The green box represents the synthesis of oCA via photoisomerization of the *cis*-isomer of CAG to the *trans*-isomer, followed by the release of oCA by employing β -glucosidase from almonds. The pink box represents the hydroxylation of oCA to GA using UPO2. (B) Deglycosylation of the *trans*-isomer of CAG (green) and photoisomerization of the *cis*-isomer of CAG, followed by deglycosylation. The samples containing the *cis*-isomer (1 mM) were first irradiated for 5 min with or without a photocatalyst (5 mol% – 0.5 mM) at 365 nm and subsequently subjected to the deglycosylation reaction. Conditions of the deglycosylation: *trans*-CAG or *cis*-CAG (1 mM), β -glucosidase (0.2 mg mL⁻¹), 100 mM NaOAc (pH 5.5), 20–24 h, 35 °C.

289 kJ mol⁻¹)^[50] or xanthone ($E_T=310$ kJ mol⁻¹)^[50] resulted in successful isomerization, resulting in a mixture of *cis*- and *trans*-isomers, according to TLC analysis. By varying the concentration of the photosensitizers, 0.36–0.55 mM oCA was formed from 1 mM *cis*-CAG following photoisomerization and deglycosylation (Figure 3B).

In summary, an efficient biosynthetic route for the synthesis of GA was developed in this study. The deglycosylation of CAG using β -glucosidase from almonds gave oCA in quantitative yield. The key step, the hydroxylation of oCA in the *para*-position relative to the existing hydroxyl group, was achieved using the unspecific peroxxygenase UPO2, which showed, despite its name, remarkable selectivity for mono-hydroxylation, obtaining GA in excellent yields. The feasibility of this route was further demonstrated by preparative scale reactions, where oCA and GA could be obtained via simple extractive workups in excellent yields. This biosynthetic pathway requires only H₂O₂ as a reagent, and chemicals and waste are kept to a minimum, aligning with global trends for greener chemical production methods.

Acknowledgements

This work was financially supported by the European Union's Horizon 2020 research and innovation programme RADICALZ (Grant number: 101000560). Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interests

All authors filed a patent application. KS is an employee of AnalytiCon Discovery GmbH.

References

- [1] R. Ullrich, M. Hofrichter, *Cell. Mol. Life Sci.* **2007**, *64*, 271–293.
- [2] P. R. Ortiz de Montellano, *Chem. Rev.* **2010**, *110*, 932–948.
- [3] L. Cheng, H. Wang, H. Cai, J. Zhang, X. Gong, W. Han, *Science* **2021**, *374*, 77–81.
- [4] H. Long, T.-S. Chen, J. Song, S. Zhu, H.-C. Xu, *Nat. Commun.* **2022**, *13*, 3945.
- [5] D. A. Alonso, C. Nájera, I. M. Pastor, M. Yus, *Chem. Eur. J.* **2010**, *16*, 5274–5284.
- [6] R. V. Ottenbacher, E. P. Talsi, K. P. Bryliakov, *Appl. Organomet. Chem.* **2020**, *34*, e5900.
- [7] B. R. Albuquerque, S. A. Heleno, M. B. P. P. Oliveira, L. Barros, I. C. F. R. Ferreira, *Food Funct.* **2021**, *12*, 14–29.
- [8] H. Zhou, B. Wang, F. Wang, X. Yu, L. Ma, A. Li, M. T. Reetz, *Angew. Chem. Int. Ed.* **2019**, *58*, 764–768.
- [9] D. Holtmann, M. W. Fraaije, I. W. C. E. Arends, D. J. Opperman, F. Hollmann, *Chem. Commun.* **2014**, *50*, 13180–13200.
- [10] R.-J. Li, K. Tian, X. Li, A. R. Gaikawari, Z. Li, *ACS Catal.* **2022**, *12*, 5939–5948.
- [11] J. Sucharitakul, C. Tongsook, D. Pakotiprapha, W. J. H. van Berkel, P. Chaiyen, *J. Biol. Chem.* **2013**, *288*, 35210–35221.
- [12] T. Hiromoto, S. Fujiwara, K. Hosokawa, H. Yamaguchi, *J. Mol. Biol.* **2006**, *364*, 878–896.
- [13] W. A. Hareland, R. L. Crawford, P. J. Chapman, S. Dagley, *J. Bacteriol.* **1975**, *121*, 272–285.
- [14] F. Lorquin, F. Ziarelli, A. Amouric, C. Di Giorgio, M. Robin, P. Piccerelle, J. Lorquin, *Sci. Rep.* **2021**, *11*, 8538.
- [15] M. Hofrichter, R. Ullrich, *Biocatal. Biotransformation Bioinorg. Chem.* **2014**, *19*, 116–125.
- [16] Y. Wang, D. Lan, R. Durrani, F. Hollmann, *Biocatal. Biotransformation Bioinorg. Chem.* **2017**, *37*, 1–9.
- [17] M. Hobisch, D. Holtmann, P. Gomez de Santos, M. Alcalde, F. Hollmann, S. Kara, *New Trends Ind. Biocatal.* **2021**, *51*, 107615.
- [18] K. Bangert, A. Swoboda, S. Vrabl, H. Rudalija, M. Lazzarotto, S. Payer, A. Glieder, C. A. M. R. van Slagmaat, S. M. A. De Wildeman, W. Kroutil, *Green Chem.* **2024**, *26*, 3183–3189.
- [19] A. C. Ebrecht, T. M. Mofokeng, F. Hollmann, M. S. Smit, D. J. Opperman, *Org. Lett.* **2023**, *25*, 4990–4995.
- [20] B. Melling, T. Mielke, A. C. Whitwood, T. J. C. O'Riordan, N. Mulholland, J. Cartwright, W. P. Unsworth, G. Grogan, *Chem Catal.* **2024**, *4*, DOI: 10.1016/j.che-cat.2023.100889.
- [21] U. René, N. Jörg, S. Katrin, S. Jörg, H. Martin, *Appl. Environ. Microbiol.* **2004**, *70*, 4575–4581.
- [22] A. Karich, M. Kluge, R. Ullrich, M. Hofrichter, *AMB Express* **2013**, *3*, 5.
- [23] K. Barková, M. Kinne, R. Ullrich, L. Hennig, A. Fuchs, M. Hofrichter, *Tetrahedron* **2011**, *67*, 4874–4878.
- [24] E. Aranda, R. Ullrich, M. Hofrichter, *Biodegradation* **2010**, *21*, 267–281.
- [25] F. Schmitz, K. Koschorreck, F. Hollmann, V. B. Urlacher, *React. Chem. Eng.* **2023**, *8*, 2177–2186.
- [26] N. Razzaghi-Asl, J. Garrido, H. Khazraei, F. Borges, O. Firuzi, *Curr. Med. Chem.* **2013**, *20*, 4436–4450.
- [27] M. Hosny, Z. Abdel-Aziz, M. M. El-aasser, M. Zhran, *Al-Azhar J. Pharm. Sci.* **2014**, *49*, 104–116.
- [28] T.-H. Chuang, H.-H. Chan, T.-S. Wu, C.-F. Li, *Molecules* **2011**, *16*, 9331–9339.
- [29] Atta-ur-Rahman, M. Shabbir, S. Ziauddin Sultani, A. Jabbar, M. Iqbal Choudhary, *Phytochemistry* **1997**, *44*, 683–685.
- [30] W.-Y. Qi, N. Ou, X.-D. Wu, H.-M. Xu, *Chin. J. Nat. Med.* **2016**, *14*, 789–793.
- [31] A. M. M. Maia, A. Pessoa-Junior, I. C. Roberto, *Ind. Crops Prod.* **2023**, *201*, 116914.
- [32] F. Chemat, M. Abert-Vian, A. S. Fabiano-Tixier, J. Strube, L. Uhlenbrock, V. Gunjevic, G. Cravotto, *TrAC Trends Anal. Chem.* **2019**, *118*, 248–263.

- [33] W. Zhang, B. D. Ames, C. T. Walsh, *Biochemistry* **2011**, *50*, 5401–5403.
- [34] S. Grüşchow, J. C. Sadler, P. J. Sharratt, R. J. M. Goss, *ChemBioChem* **2020**, *21*, 417–422.
- [35] S. L. Lovelock, N. J. Turner, *Nat. Eng. Artif. Biocatal. Org. Synth.* **2014**, *22*, 5555–5557.
- [36] Y. Brack, C. Sun, D. Yi, U. T. Bornscheuer, *ChemBioChem* **2022**, *23*, e202200062.
- [37] S. D. Sarker, L. Nahar, in *Prog. Chem. Org. Nat. Prod. 106* (Eds.: A. D. Kinghorn, H. Falk, S. Gibbons, J. Kobayashi), Springer International Publishing, Cham **2017**, pp. 241–304.
- [38] F. Wu, Z. Duan, P. Xu, Q. Yan, M. Meng, M. Cao, C. S. Jones, X. Zong, P. Zhou, Y. Wang, K. Luo, S. Wang, Z. Yan, P. Wang, H. Di, Z. Ouyang, Y. Wang, J. Zhang, *Plant Biotechnol. J.* **2022**, *20*, 592–609.
- [39] L. Rotilio, A. Swoboda, K. Ebner, C. Rinnofner, A. Glieder, W. Kroutil, A. Mattevi, *ACS Catal.* **2021**, *11*, 11511–11525.
- [40] M. Ramirez-Escudero, P. Molina-Espeja, P. Gomez de Santos, M. Hofrichter, J. Sanz-Aparicio, M. Alcalde, *ACS Chem. Biol.* **2018**, *13*, 3259–3268.
- [41] M. Sugumaran, J. J. Evans, *J. Funct. Biomater.* **2023**, *14*, DOI: 10.3390/jfb14090449.
- [42] M. Friedman, H. S. Jürgens, *J. Agric. Food Chem.* **2000**, *48*, 2101–2110.
- [43] P. L. Pasquet, D. Julien-David, M. Zhao, M. Villain-Gambier, D. Trébouet, *Food Biosci.* **2024**, *57*, 103586.
- [44] A. Becker, D. Böttcher, W. Katzer, K. Siems, L. Müller-Kuhr, U. T. Bornscheuer, *ChemCatChem* **2020**, *12*, 4084–4089.
- [45] F. Ieri, P. Pinelli, A. Romani, *Food Chem.* **2012**, *135*, 2157–2162.
- [46] M. Zaynab, J. Khan, R. Al-Yahyai, M. Saddar, S. Li, *Toxicon* **2024**, *250*, 108118.
- [47] Z.-J. Li, L. Cai, R.-F. Mei, J.-W. Dong, S.-Q. Li, X.-Q. Yang, H. Zhou, T.-P. Yin, Z.-T. Ding, *Tetrahedron Lett.* **2015**, *56*, 7197–7200.
- [48] J. B. Metternich, R. Gilmour, *J. Am. Chem. Soc.* **2015**, *137*, 11254–11257.
- [49] R. W. Chambers, D. R. Kearns, *Photochem. Photobiol.* **1969**, *10*, 215–219.
- [50] N. A. Romero, D. A. Nicewicz, *Chem. Rev.* **2016**, *116*, 10075–10166.