A Journal of the Gesellschaft Deutscher Chemiker

Angewandte International Edition

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Accepted Article

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To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201802176
Angew. Chem. 10.1002/ange.201802176

Link to VoR: http://dx.doi.org/10.1002/anie.201802176
http://dx.doi.org/10.1002/ange.201802176

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Chemo-Enzymatic Total Synthesis of Oxosorbicillinol, Sorrentanone, Rezishanones B and C, Sorbicatechol A, Bisvertinolone and (+)-Epoxysorbicillinol

Dedicated to Professor Dr. Wolfgang A. Hermann on the occasion of his 70th birthday

Anna Sib and Tobias A. M. Gulder*

Abstract: *The sorbicillinoids are a large family of fungal natural products, many of which possessing highly challenging molecular architectures. Depending on their individual structures they exhibit strong biological activities ranging from radical scavenging and anti-infective properties to cytotoxicity. Despite the resulting strong biomedical potential of these natural products and the interest of the total synthetic community owing to their fascinating structures, many sorbicillinoids are currently not synthetically accessible, thus hampering in depth biological characterization and structural diversification. Using recombinant oxidoreductase SorbC and readily accessible sorbicillin-type synthetic precursors, we developed enantioselective, one-pot chemo-enzymatic routes to a broad range of sorbicillinoids, thereby establishing the first total syntheses of oxosorbicillinol, sorrentanone, rezishanones B and C, sorbicatechol A, bisvertinolone, and (+)-epoxysorbicillinol.*

The sorbicillinoid family of fungal natural products stands out due to its exceptional diversity of molecular structures and selective biological activities, despite all members being derived of the simple polyketide sorbicillin (**1**).[1] Aside from structural analogs of **1**, e.g., characterized by slightly different substitution patterns at the aromatic ring system or varying saturation of the sorbyl side chain, the sorbicillinoids biosynthetically originate from oxidative dearomatization of **1** to sorbicillinol (**2**) by the monooxygenase SorbC.[2] This transformation is achieved with perfect regio- and stereocontrol.[3] Sorbicillinol (**2**) contains a highly reactive *cis* diene that is concurrently part of an activated Michael acceptor system. Much of the structural diversity of the sorbicillinoids results from dimerization of **2** guided by its inherent reactivity. This leads, i.a., to the formation of the radical scavenger bisorbicillinol (**3**)[4] by Diels-Alder cycloaddition, or of the inhibitor of the prostaglandin-H-synthase-2 trichodimerol (**4**)[5] by a Michael-addition / ketalization sequence (Figure 1). In addition, Nature is capable of taming the high reactivity of **2** sufficiently to also allow alternative functionalization pathways to occur. For example, a Diels Alder reaction with a vinylphenyl dienophile leads to sorbicatechol A (**5**),[6] while epoxidation of **2** leads to (+)-epoxysorbicillinol (**6**),[7] and a Michael-addition with an alanine-derived *C*-nucleophile paves the way for sorbicillactone A (**7**) formation.[8] The catalytic activity of a single enzyme, SorbC, thus gives rise to the complete metabolic diversity of the sorbicillinoid natural product family. This enzyme therefore bears exceptional potential as a biocatalytic tool in the total synthesis of these structurally challenging natural products starting from a single, readily available synthetic precursor molecule, sorbicillin (**1**). This is particularly rewarding due to the strong and selective biological properties that can be found in the sorbicillinoid natural product family. For example, **5** exhibits promising antiviral properties against the influenza A virus (H1N1), with IC_{50} values of 85 µM, thus in the range of the commercial antiviral agent ribavirin used as a control with an IC_{50} of 84 µM.[6] Sorbicillactone A (**7**) not only possesses strong anti-HIV activity by inhibiting viral protein expression and protecting human T lymphocytes against the cytopathic effect of HIV-1, but also has selective activity against L5178y murine leukemic lymphoblasts at an IC_{50} of 2.2 µg/mL.[8] Developing fast, unified synthetic approaches targeting this promising natural product family is thus of importance to facilitate further investigations into their biomedical value.

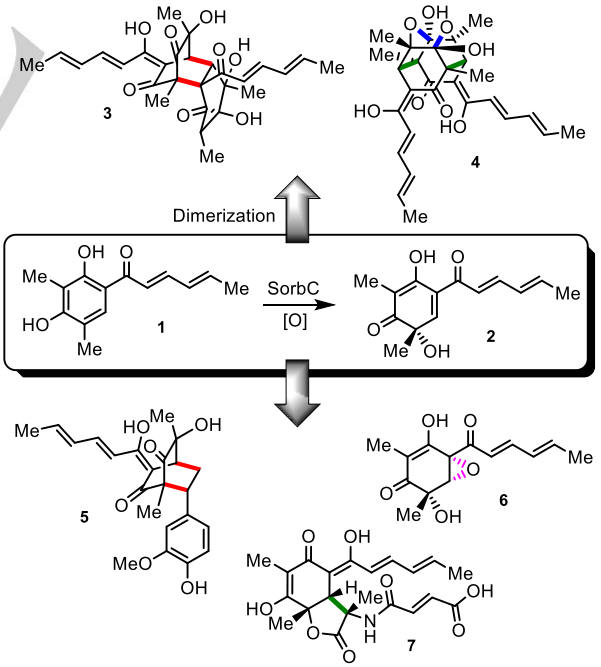

Figure 1. Structural diversity of sorbicillinoid natural products derived of the oxidative dearomatization of **1** to **2** with subsequent dimerization to give bisorbicillinoids, e.g., **3** and **4**, or further functionalization reactions, e.g., leading to **5**-**7**. Bond formation is achieved by Diels-Alder (red bonds), Michael-Addition (green bonds), ketalization (blue bonds), and oxidation (pink bonds) reactions.

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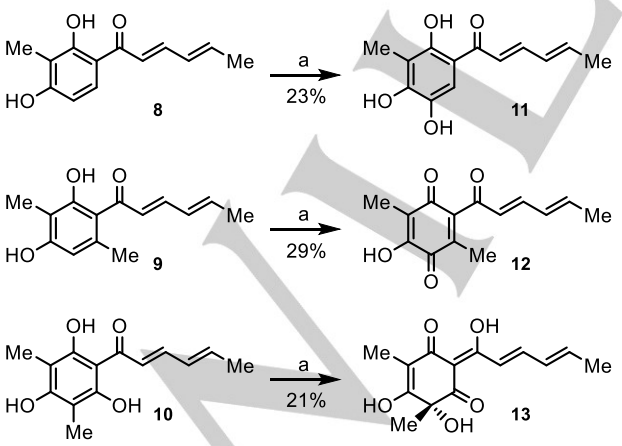
We have recently shown that SorbC can indeed be utilized for the fast and stereoselective total synthesis of bisorbicillinoids resulting from dimerization of **2**, e.g., leading to **3** and **4**, or from Diels-Alder reaction of unreacted **1** with **2**, e.g., facilitating the first total synthesis of sorbiquinol.[3] The enzyme also accepted structural analogs of **1** as substrates, allowing for the synthesis of unnatural bisorbicillinoid derivatives.[3] The catalytic activity of SorbC towards a larger set of substrates was recently evaluated by Narayan and coworkers, also including the monooxygenases TropB from stipitatonic acid biosynthesis[9] and AzaH from azaphilone biosynthesis[10] offering different site- and stereoselectivity. This work nicely showcases the broad synthetic potential of these biocatalysts for oxidative dearomatization of α-acylated or -formylated phenols.[11]

Following our interest in utilizing SorbC for the total synthesis of functionalized sorbicillinoid natural products that do not solely derive from the reaction of **2** with **1** or **2**, we tested the catalytic activity of SorbC towards a set of further monomeric sorbicillin derivatives **8**-**10** with different substitution pattern at the aromatic ring system. These substrates were prepared by $AlCl_3$-promoted Friedel-Crafts acylation of the corresponding phenols with sorbic acid chloride (see Supporting Information). Interestingly, the natural product demethylsorbicillin (**8**)[12] was selectively oxidized to triol **11** in 23 % yield,[13] while compound **9**, in which one methyl group is moved from C-5 to C-6 relative to sorbicillin (**1**), is oxidized to the corresponding para quinone **12** in 29 % yield (Scheme 1), a compound know as natural product sorrentanone from *Penicillium chrysogenum*.[14] When the hexa-substituted phloroglucinol **10** was used as a substrate, conversion to the natural product oxosorbicillinol (**13**)[12] was achieved in 21% yield (*er* = 95:5, see Supporting Information). In contrast to the highly reactive sorbicillinol (**2**), all oxidation products **11**-**13** from these transformations are stable compounds. Exemplarily for the production of **12** we furthermore investigated in situ NADH co-factor regeneration using glucose dehydrogenase Gdh.[15] This facilitated the reduction of the amount of NADH from approx. 1.2 equivalents to 0.2 equivalents without affecting product yields (see Supporting Information).



Scheme 1. Oxidation of sorbicillin analogs **8**-**10** to give triol **11** and the natural products sorrentanone (**12**) and oxosorbicllinol (**13**), respectively. a: phosphate buffer (50 mM, pH = 8.0), SorbC, NADH, room temperature; acetone was used as co-solvent (cs) for solubilisation of substrates **8**-**10**.

The oxidative dearomatization of **10** by SorbC did not only conclude the first synthesis of the natural product **13**, but also promised direct access to the structurally challenging bisvertinolone (**14**), as this natural product likely results from a biosynthetic Michael-Addition / ketalization sequence of **2** with **13**.[16] As our previous work only permitted the fusion of sorbicillinol (**2**) with itself or sorbicillin (**1**),[3] a method for the extraction of **2** from the enzymatic transformation followed by a controlled reaction setup for further functionalization was required to pave the way for the chemo-enzymatic synthesis of further diversified sorbicillinoids such as **14**. Given the increased stability of **2** in polar solvents, such as H_2O or DMF, the use of significant amounts of DMF as co-solvent in the aqueous enzymatic transformations followed by its extraction from the aqueous phase with CH_2Cl_2 and the fast removal of only the CH_2Cl_2 under reduced pressure was thought to give access to semi-purified **2** in DMF solution for further functionalization chemistry. While the formation of the dimeric bisorbicillinol (**3**) and other minor products resulting from the inherently high reactivity of **2** towards dimerization can not entirely be prevented during this work-up procedure, this strategy indeed enables the controlled reaction of **2** with alternative building blocks. This facilitated the first total synthesis of bisvertinolone (**14**) in 20 % isolated yield by treatment of the DMF solution of **2** with oxosorbicillinol (**13**) and pyridine, promoting a highly selective Michael-Addition / ketalization sequence (Scheme 2).

The extension of this methodology to the synthesis of various other sorbicillinoids, derived by Diels-Alder reaction of **2** with different dienophiles, was subsequently evaluated. An interesting set of target compounds were the two reported ether derivatives sorbivineton[8a] and rezishanone C.[17] However, as recently shown by synthetic work by Yan et al.,[18] the structures of these compounds are indeed identical and correspond to **17**. This product was obtainable within our work by addition of ethylvinylether (**15**) to **2** in DMF solution to give **17** in 29% yield (Scheme 2). While the above-mentioned synthesis of the unnatural *ent*-**17** by Yan et al. required > 20 individual synthetic steps,[18] our biocatalytic approach delivered **17** in a single step from sorbicillin (**1**), with the latter being accessibly in a simple additional three-step synthetic sequence.[3] Rezishanone B (**18**)[17b] was likewise accessible in 32% yield following this approach by using *n*-butyl vinyl ether (**16**) as the dienophile. As an alternative to the use of DMF and its extraction with CH_2Cl_2, the application of acetone as the co-solvent during the enzymatic synthesis of **2** with subsequent CH_2Cl_2 extraction followed by the direct addition of the desired dienophile to the combined organic phases and slow evaporation of the solvent was also feasible for non-volatile enes. Using 4-ethenyl-2-methoxyphenol (**19**) as the reaction partner for **2** thereby facilitated the first total synthesis of the anti-viral sorbicatechol A (**5**)[6] in 30% yield. Interestingly, while the NMR data of synthetic **5** perfectly matched the data reported for isolated **5**, optical rotation values were in disagreement, including opposite signs. This discrepancy was resolved by comparison of the CD spectra, revealing a perfect match of synthetic with isolated **5** (see Supporting Information). As the optical rotations of unnatural analogs of **5** with variations in the aromatic portion (see below) were in a similar range, an incorrect value reported for **5** in the original isolation paper is likely.

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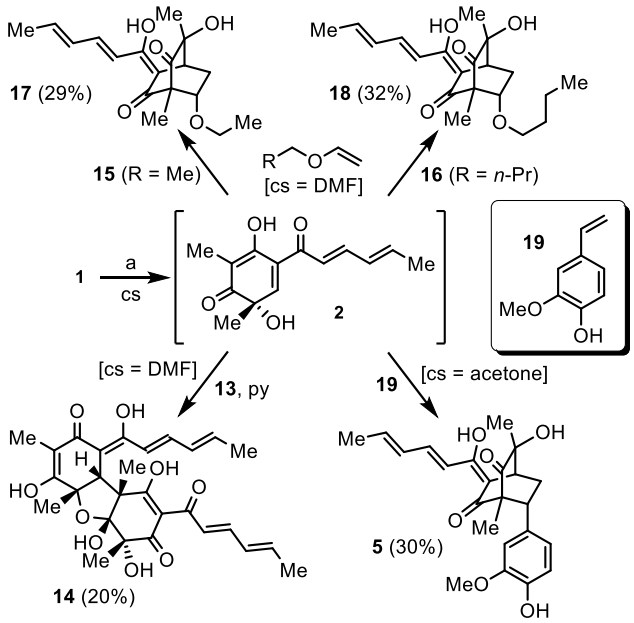
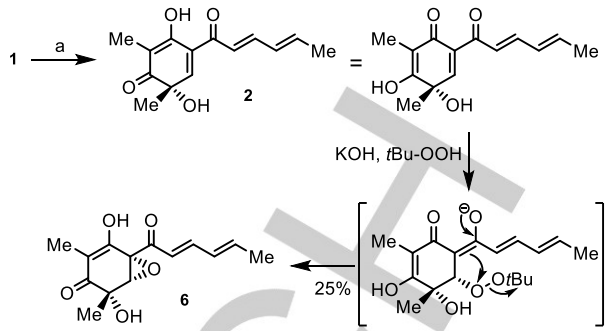


Scheme 2. Chemo-enzymatic total syntheses of bisvertinolone (**14**), rezishanone C (**17**), rezishanone B (**18**) and sorbicatechol A (**5**). a: cf. Scheme 1; cs = co-solvent.

To further demonstrate the applicability of this chemo-enzymatic approach, we set out to prepare a set of unnatural sorbicillinoids derived of Diels-Alder cycloaddition of **2** with vinyl ethers or styrens. Using phenyl vinyl ether, *tert*-butyl vinyl ether, 1-methyl-2-vinylbenzene or 4-vinylaniline readily gave the expected addition products **20**-**23** in 15-30% yield (see Supporting Information for details). These results emphasize that our chemo-enzymatic approach is suitable for the fast generation of mg quantities of sorbicillinoid Diels-Alder analogs sufficient for structure-activity relationship studies.

Encouraged by these results we were interested to probe if functionalization of **2** in stabilizing, polar solution is also possible using non-sorbicillinoid, external nucleophiles. An interesting target structure to this end was (+)-epoxysorbicillinol (**6**),[7] which is derived from regio- and stereoselective epoxidation of one of the double bonds in the cyclohexene unit of **2**. Despite considerable interest in the synthesis of **6**, the only two currently existing total synthetic routes by Pettus et al.[19] and Wood et al.[20] lead to a mixture of the enantiomers (+/-)-epoxysorbicillinol (**6**). We envisioned that functionalization of the desired double bond in **2** should selectively be possible by conjugate addition due to its double activation (Scheme 3). Introduction of the required epoxy function should thus be possible by conducting a Weitz-Scheffer epoxidation using KOH and *t*-butylperoxide. The tertiary alcohol function in **2** could thereby direct the potassium *t*-butylperoxide to preferentially attack from the same side by pre-complexation. This would allow chirality transfer from the enzymatically installed stereogenic center to the epoxide unit. As a consequence, this should preferentially lead to the desired configuration at the newly formed stereogenic centers. When a DMF solution containing **2** was cooled to 0°C and treated with KOH and *t*-butylperoxide, the exclusive formation of (+)-epoxysorbicillinol (**6**) in 25% yield as a single stereoisomer was indeed achieved.



Scheme 3. Chemo-enzymatic, stereoselective total synthesis of (+)-epoxysorbicillinol (**6**). a: cf. Scheme 1; cs = DMF.

Taken together, we have developed a chemo-enzymatic strategy for the stereoselective total synthesis of diverse sorbicillinoids and unnatural analogs that involves the partial purification of the highly reactive **2** in DMF solution to enable subsequent cyclo- and conjugate addition chemistry. Using different dienophiles, this paved the way for the first total syntheses of bisvertinolone (**14**), rezishanones C (**17**) and B (**18**), as well as sorbicatechol A (**5**). Utilizing a Weitz-Scheffer epoxidation furthermore gave the first stereoselective access to (+)-epoxysorbicillinol (**6**). In combination with our previous establishment of oxidative dearomatization / dimerization sequences towards bisorbicillinoids,[3] this chemo-enzymatic toolbox provides highly streamlined access to most sorbicillinoids. The application of this approach to the synthesis of focused compound libraries for the sorbicillinoid congeners with the most promising biological activities will now facilitate the evaluation of the biomedical potential of this natural product family. This work is currently in progress in our laboratory.

Acknowledgements

We thank the groups of Prof. Dr. S. Sieber (TU Munich) for measuring HRMS data and of Prof. Dr. Tanja Gulder (TU Munich) for providing some of the chemical starting materials. We are very grateful to the DFG for generous financial support of this work (GU 1233/1-1 and the Center for Integrated Protein Science Munich CIPSM).

Conflict of interest

The authors declare no conflict of interest.

Keywords: biocatalysis • enzyme catalysis • sorbicillinoids • natural products • total synthesis

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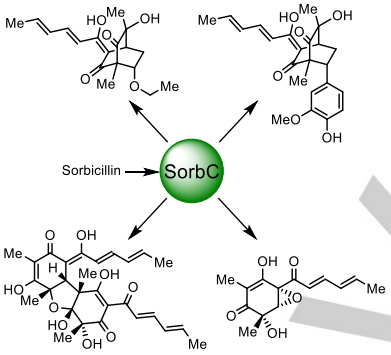
Sorbicillinoids made easy. The first stereoselective total syntheses of the structurally diverse sorbicillinoids oxosorbicillinol, sorrentanone, rezishanones B and C, sorbicatechol A, bisvertinolone and (+)-epoxy-sorbicillinol are established applying a unifying chemo-enzymatic approach that involves a regio- and enantio-selective oxidative de-aromatization of sorbicillin-type precursors harnessing the catalytic activity of the mono-oxygenase SorbC.



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