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New Sesquiterpene Oxidations with CYP260A1 and CYP264B1 from *Sorangium cellulosum* So ce56

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Sesquiterpenes are natural products derived from the common precursor farnesyl pyrophosphate (FPP) but are highly diverse in structure and function. Cytochrome P450 enzymes (P450s) exhibit the unique ability to introduce molecular oxygen into non-activated C–H bonds. In plant biosynthetic pathways, P450s commonly derivatize sesquiterpene hydrocarbons. However, the potential of bacterial P450s for terpene derivatization is still underinvestigated. This work compares the substrate specificities and regioselectivities of the sesquiterpene hydroxylases CYP260A1 and CYP264B1 from myxobacterium *Sorangium cellulosum* So ce56. Four tested substrate classes (eremophilanes, humulanes, caryophyllanes, and cedranes) were converted by both P450s. The achievable variety of oxidations is demonstrated on the model substrates (+)-nootkatone and zerumbone. Increasing the number of functionally investigated P450s, this study represents a step towards the selective derivatization of sesquiterpenes.

Introduction

Sesquiterpenes are a group of natural products biosynthesized either through the mevalonate pathway or the methylerythritol pathway.[1] Although all sesquiterpenes contain 15 carbon atoms (C_{15}) and derive from the common precursor farnesyl pyrophosphate (FPP) through bioconversion catalyzed by terpene synthases (TPSs),[2] they display highly diverse branched, mono-, bi-, and tricyclic hydrocarbon skeletons.[3] Physiological derivatization is conducted by other enzymes, most often cytochromes P450 (P450s),[4] so that 89.5% of all known sesquiterpenes are derivatized.[5] P450s are external monooxygenases. They selectively introduce molecular oxygen into non-activated C–H bonds, and this makes them very powerful for applications in synthesis as a complement to chemical methods.[6]

Lightly derivatized sesquiterpenes are volatile and serve for plants as a means of chemical communication either for defense or for attraction of pollinators.[7] Mankind has discovered plant sesquiterpenoids as highly valuable flavors and fragrances,[8] and multiply oxidized derivatives as antitumor[9] or antimalaria agents.[10]

Recently, bacterial sesquiterpenes have been studied more intensely;[11–12] their physiological functions remain a topic of discussion.[12] Nevertheless, bacterial sesquiterpene biosynthesis pathways have been identified as analogous to those in plants, including conversion of FPP by TPSs and derivatization of the hydrocarbons by P450s.[11,13] In a similar way, smaller monoterpenes (C_{10}) and bigger di- (C_{20}) and triterpenes (C_{30}) are synthesized.[1]

Obtaining valuable terpene intermediates is an important challenge, because they often do not accumulate in the host organisms. Chemical synthesis is also often inefficient or unselective. An increasing knowledge of biosynthesis pathways has thus led to the evolution of synthetic biology as a tool for the production of valuable terpene compounds.[14] Crucial steps in the synthesis of oxidized sesquiterpenes are catalyzed by P450s.[5] Consequently, whole-cell biocatalysts are designed for efficient P450-mediated derivatization of sesquiterpenes. The hydrocarbon precursors or low-priced mevalonate pathway intermediates are used as substrates.[15–16] P450s of microbial origin have shown to perform commercially interesting terpene oxidations.[17] For biosynthetic purposes they could represent an efficient alternative to plant P450s, because they show generally high expression rates in *Escherichia coli*, and their combinations with electron-transfer proteins are readily available as bacterial whole-cell catalysts.[17]

Previously, we have identified the CYP106 family from *Bacillus megaterium* as di- and triterpene hydroxylases.[18–20] We have also revealed the biosynthetic potential of CYP105A1 from *Streptomyces griseolus*. It mediates the direct conversion of the diterpene resin abietic acid to its 15-hydroxy derivative, a key precursor of 15-hydroperoxyabietic acid, thus allowing a nine-step chemical synthesis to be avoided.[21]

Because bacterial P450s are able to perform these reactions, we have previously established P450-based *E. coli* whole-cell catalysts for in vivo terpene oxidations,[22–24] and have optimized the electron transfer onto bacterial P450s.[25] Seeking for

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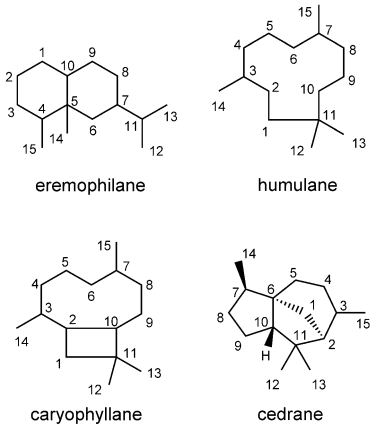
new versatile sesquiterpene-derivatizing P450s, we turned to the myxobacterial strain *Sorangium cellulosum* So ce56. It is commonly found in upper soil fractions close to plant roots and possesses one of the largest bacterial genomes (13.1 Mbp), including 21 functional P450 genes with great potential for biosynthetic applications.[26]

Among them we found two sesquiterpene-converting enzymes: CYP260A1 oxidizes the grapefruit flavor (+)-nootkatone,[29] whereas CYP264B1 is a native (+)-eremophilene hydroxylase,[27] which was also shown to convert other sesquiterpenes of different parent structures.[30] While further exploiting the sesquiterpene derivatization potential of these P450s, we treated them with a broad range of sesquiterpenes for conversion. The current work reveals CYP260A1 and CYP264B1 to be versatile sesquiterpene oxidases, and compares their substrate specificities and selectivities. As substrates we chose monocyclic humulanes and their bicyclic caryophyllane derivatives, bicyclic eremophilanes, and tricyclic cedranes (Scheme 1). It was demonstrated that CYP260A1 efficiently converts oxygenated sesquiterpenes and that CYP264B1 regioselectively introduces allylic alcohol functions.



Scheme 1. Hydrocarbon skeletons of the sesquiterpenes tested for CYP260A1- and CYP264B1-mediated derivatization.

Results and Discussion

For conversions of a broad range of sesquiterpene substrates, the (+)-nootkatone oxidase CYP260A1[29] and the native (+)-eremophilene hydroxylase CYP264B1[27] were selected from a range of 21 functional *S. cellulosum* So ce56 cytochromes P450.[26] The readily available *E. coli*-based P450 whole-cell catalysts allow cost-efficient conversions on a milligram scale for structural analysis of the major products by NMR spectroscopy, in addition to GC/MS.[25,27,31]

Four structural classes of sesquiterpene substrates (Scheme 1) were chosen for conversion tests with CYP260A1 and CYP264B1. Pairs of substrates were used from the classes of eremophilanes, humulanes, and cedranes. The eremophilanes (+)-nootkatone (Scheme 2, **1**) and (+)-valencene (**4**), as well as the humulanes zerumbone (**6**) and α-humulene (**12**), represent pairs of hydrocarbons and their corresponding ketones. The cedranes (+)-3(15)-cedren-4-ol (**16**) and α-cedrene (**21**) differ in the hydroxy function at C-4 and in the position of a double bond: between carbon atoms 3 and 4 or 3 and 15, respectively. β-Caryophyllene (**14**) is related to **12**; however, it is less flexible due to the additional intramolecular bond between C-2 and C-10, which forms a strained cyclobutane ring.

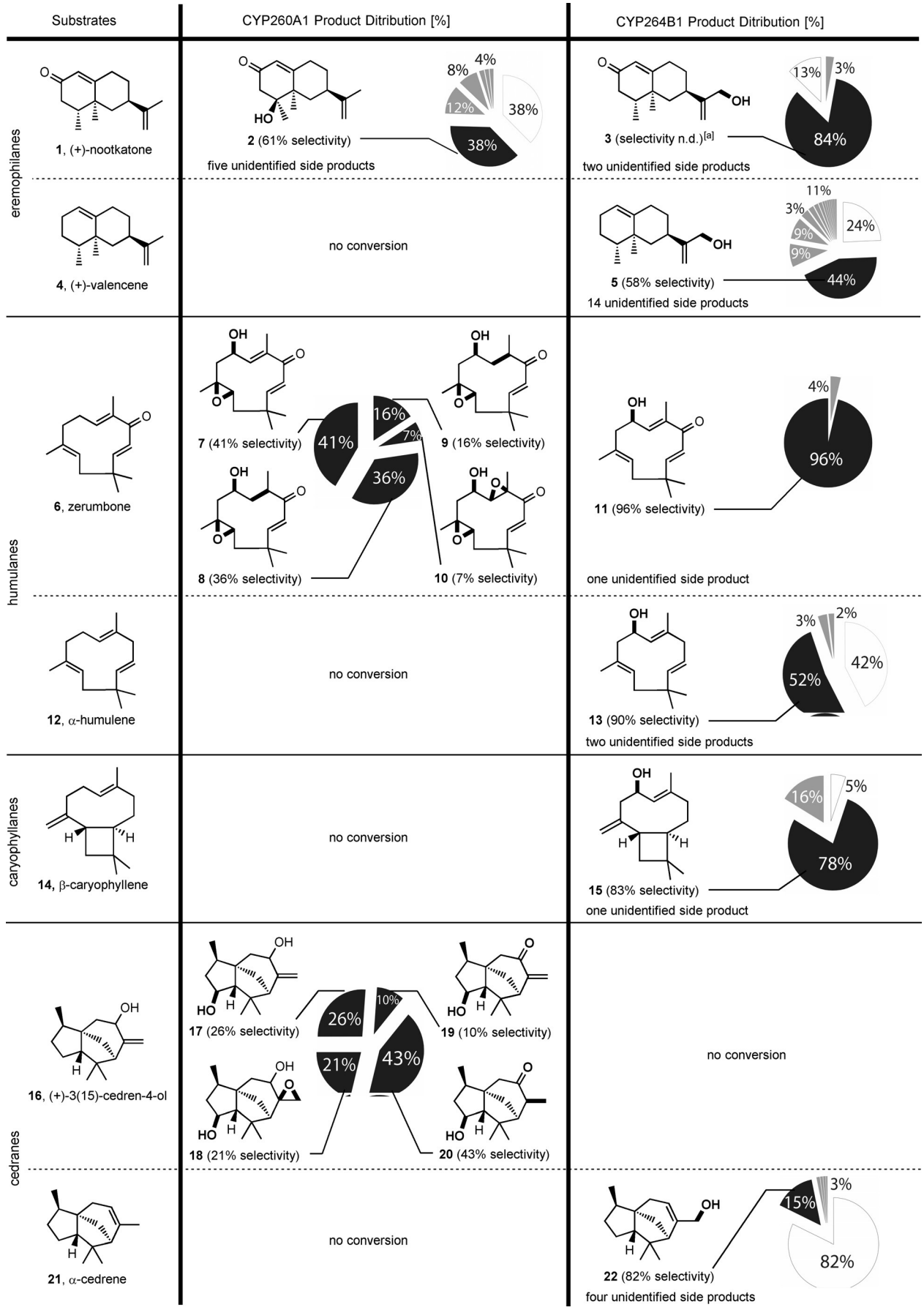
CYP260A1-dependent conversion of sesquiterpenes

CYP260A1 converts the sesquiterpene ketones (+)-nootkatone (Scheme 2, **1**) and zerumbone (**6**), as well as the allylic sesquiterpene alcohol (+)-3(15)-cedren-4-ol (**16**). Whereas **1** is 62% consumed, the other two substrates are converted completely under the experimental conditions (Scheme 2, column 2). The tested non-oxidized hydrocarbon sesquiterpenes (compounds **4**, **12**, **14**, and **21**) are not converted by this P450.

The extract from the whole-cell conversion of **1** by CYP260A1 contains six products. Its main product was determined to be 4-hydroxynootkatone (**2**, 61% selectivity; Scheme 2, line 1). Addition of (+)-valencene (**4**) to the whole-cell catalyst gave no conversion. The humulane-type zerumbone (**6**) was converted quantitatively into four different products. Each of these oxidation products bears a hydroxy function at the C-5 atom and an epoxide group between the atoms C-2 and C-3 (compounds **7**–**10**; Scheme 2, line 3). Two of the products, **8** and **9**, are isomers, with a saturated bond between C-6 and C-7. In the fourth product, compound **10**, this initial double bond is epoxidized. The hydrocarbon α-humulene (**12**) and the related β-caryophyllene (**14**) are not oxidized by CYP260A1. From the group of cedrane-type sesquiterpenes, CYP260A1 oxidized (+)-3(15)-cedren-4-ol (**16**) to four products, all hydroxylated at position 9 (compounds **17**–**20**; Scheme 2, line 6). Additionally, the conversion of **16** by CYP260A1 affected the double bond between C-3 and C-15, which was either epoxidized in compound **18** or reduced in compound **20**. The allylic alcohol function of **16** at C-4 can be oxidized to a ketone, in compounds **19** and **20**.

Overall, CYP260A1 catalyzes sesquiterpene conversion for eremophilane-, humulane-, and cedrane-type structures, into a multitude of products in each case. The presence of oxy functions seems to be imperative, because non-derivatized hydrocarbon sesquiterpenes **4**, **12**, **14**, and **21** are not oxidized. Under the defined reaction conditions, the substrates are efficiently converted and often oxidized at different positions (compounds **7**–**10** and **17**–**20**). The selectivities are rather low, with 61% selectivity for the oxidation of **1** to **2** being the high-

Scheme 2. Overview of the sesquiterpene conversions mediated by CYP260A1 and CYP264B1: structures of the tested sesquiterpene substrates (including names) and the determined products (including selectivities). The product structures were determined by NMR and confirmed by GC/MS. Functionalizations introduced by the P450s are represented in bold. The yields and selectivities were calculated on the basis of GC/MS and HPLC UV/Vis peak areas. Pie charts for the degrees of substrate conversion include substrates (white), structurally determined main products (black), and undetermined side products (gray). n.d.: not determined. [a] The substances **3** and another (+)-nootkatone product could not be completely separated by GC/MS: 84% refers to the sum of both products.



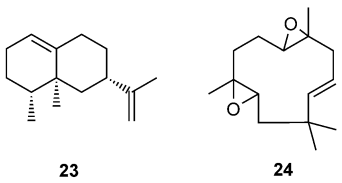
est (Scheme 2, column 2). However, it has to be taken into account that the reaction conditions have not been optimized to obtain higher selectivities.

The introduced oxy functions in the humulanes **7**–**10** and the cedranes **17**–**20** are situated at considerable distances from each other. Thus, several binding modes for the substrates and intermediate products seem to be possible. This is also the case for **1**, because four out of its six products are monohydroxylated (results not shown). The conversion of **6** is likely to begin either with hydroxylation at C-5 (compounds **7**–**10**) or with the epoxidation of the C-2=C-3 double bond (also compounds **7**–**10**). It is likely to be completed with saturation of the C-6=C-7 double bond (compounds **8** and **9**) through the epoxide intermediate **10**. Compound **16** is first hydroxylated at C-9 (compounds **17**--**20**); further reaction steps involving oxidation of the 4-hydroxy group (compounds **19** and **20**) as well as epoxidation and reduction of the C-3=C-15 double bond (compounds **18** and **20**) do not seem to be conducted in a defined order.

CYP264B1-dependent conversion of sesquiterpenes

In contrast to the experiments with CYP260A1, each of the tested sesquiterpenes was converted by CYP264B1, with the exception of (+)-3(15)-cedren-4-ol (Scheme 2, **16**). All of the determined products—compounds **3**, **5**, **11**, **13**, **15**, and **22**—contain allylic hydroxy groups.

CYP264B1, a native (+)-eremophilene hydroxylase, oxidized (+)-nootkatone (**1**) and its non-oxygenated precursor (+)-valencene (**4**), the C-7 epimer of (+)-eremophilene (Scheme 3, **23**). In vivo conversions of **1** produced mainly 13-hydroxynootkatone (**3**) along with two side products (Scheme 2, line 1). Compound **4** was oxidized to 15 different products, with 13-hydroxyvalencene (**5**) as the main product (58% selectivity, Scheme 2, line 2). Both of the humulane-type substrates zerumbone (**6**) and α-humulene (**12**) were hydroxylated at position C-5 with selectivities of 96 and 90%, respectively (Scheme 2, lines 3 and 4). The humulane-related β-caryophyllene (**14**) was also selectively converted into its 5-hydroxy derivative **15** (83% selectivity, Scheme 2, line 5). Relative to those of the eremophilanes, the selectivities for humulane and caryophyllane conversions are remarkably high. Even the native CYP264B1 substrate (+)-eremophilene (**23**) is converted into six different products.[27] For products obtained from both eremophilane- and humulane-type substrates, the levels of conversion were higher and more selective for the keto derivatives **1** (97% conversion, three products) and **6** (100%, two products) than for the non-oxidized hydrocarbons **4** (76%, 15 products) and **12** (58%, three products; Scheme 2, column 3). Compound **16** was the only substrate that could not be oxidized by CYP264B1. In contrast, this P450 converted α-cedrene (**21**) into its 15-hydroxy derivative **22**, introducing a primary allylic alcohol function. Steric hindrance in binding of **16** might have been due to the hydroxy group at C-4 or the terminal double bond between C-3 and C-15. This might affect the difference between the conversions of **16** and **21**.



23 **24**

Scheme 3. Sesquiterpene derivatives of the tested substrates. (+)-Eremophilene (**23**) is the native substrate of CYP264B1;[27] (9*E*)-humulene-2,3;6,7-diepoxide (**24**) has been shown to have anti-acetylcholinesterase activity.[28]

Overall, it can be stated that CYP264B1 is a highly selective sesquiterpene hydroxylase. It produces allylic alcohols (at position C-5) from the humulanes **6** and **12** and also from caryophyllene (**14**). In the cases of the eremophilane-type substrates **1** and **4** it shows increased 13-hydroxylation activity, likewise producing allylic alcohols. For these structural sesquiterpene classes, the regioselectivity of the hydroxylation does not seem to be affected by the presence of a carbonyl function or by the higher flexibility of humulanes in relation to caryophyllene. However, a carbonyl group enhances the catalytic activity of CYP264B1 towards eremophilanes and humulanes: hydrocarbon substrates **4** and **12** are converted less efficiently and less selectively than their keto derivatives **1** and **6**. For cedranes, the presence of a 4-hydroxy group and a change in a double bond (**16** in comparison with **21**) abolish the catalytic function of CYP264B1.

Comparison of CYP260A1 and CYP264B1

Both P450s—CYP260A1 and CYP264B1—have shown their versatilities in oxidizing sesquiterpenes. All of the four structural classes of sesquiterpenes employed as substrates (Scheme 1) were converted either by one or by both of the enzymes. This indicates that the active sites of the P450s form cavities large or flexible enough for the binding of these structurally diverse sesquiterpenes.

Sesquiterpenes possessing oxygen-containing functional groups (Scheme 2, compounds **1**, **6**, and **16**) serve as substrates for CYP260A1, whereas the corresponding hydrocarbons are not converted. Thus, this enzyme is likely to bind the substrates through hydrogen bonds. Its oxidation reactions show high promiscuity, producing several products in similar amounts (compounds **7**–**10** from **6**, compounds **17**–**20** from **16**) by oxidizing each substrate at different positions. Wild-type CYP260A1 therefore seems a rather poor candidate for selective sesquiterpene oxidation. However, it does hydroxylate sesquiterpenes at chemically inert positions (compound **1** at C-4, compound **16** at C-9). In analogy to previous studies with CYP106A2,[32] rational mutagenesis of CYP260A1 could be conducted to increase its selectivity. The conversions of **1**, **6**, and **16** demonstrated in this study represent suitable model reactions for this purpose.

CYP264B1 converts all of the employed substances, whether they are oxygenated or non-oxygenated hydrocarbons, except for (+)-3(15)-cedren-4-ol (**16**). The enzyme's active site seems

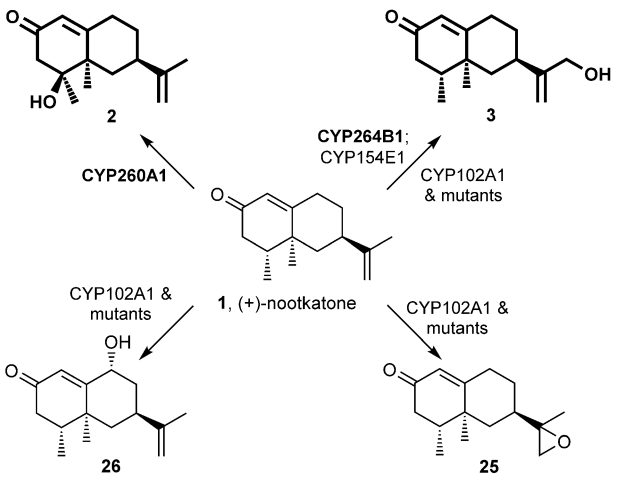
to be governed by hydrophobic residues, able to bind unpolar sesquiterpene hydrocarbons. However, the ketones **1** and **6** are converted more efficiently and more selectively than their non-oxidized parent sesquiterpenes **4** and **12**. This might be explained by hydrogen-bond-directed preorientation of the substrate ketones in the active site. A computational model of **1** bound to CYP264B1 shows binding of the carbonyl group to the backbone amide of Met286.[30] CYP264B1 shows high selectivities for C-5 hydroxylation of the humulanes **6** and **12** as well as for β-caryophyllene (**14**). Therefore, neither the 8-keto function nor the greater flexibility of humulanes in relation to the related caryophyllanes have an effect on the regioselectivity of the hydroxylation. Eremophilane-type substrates are converted into a multitude of products, mainly hydroxylated at C-13 (compounds **3** and **5**). The occurrence of some side products, which has likewise been observed for the physiological substrate (+)-eremophilene,[27] is probably due to reactions taking place in the *E. coli* whole-cell catalyst. CYP264B1-mediated in vitro hydroxylations of **1** and **4** have been shown to produce only one and four side products, respectively.[30] Thus, CYP264B1 is a selective sesquiterpene oxidase, which is able to functionalize a broad range of substrate structures. In general, sesquiterpene olefins of every hydrocarbon skeleton type should be considered as substrates for CYP264B1.

Taken together, both P450s—CYP260A1 and CYP264B1—should be considered for oxidation studies of further sesquiterpene classes. Whereas wild-type CYP264B1 is selective for allylic hydroxylations, CYP260A1 needs to be engineered to perform reactions with higher selectivities.

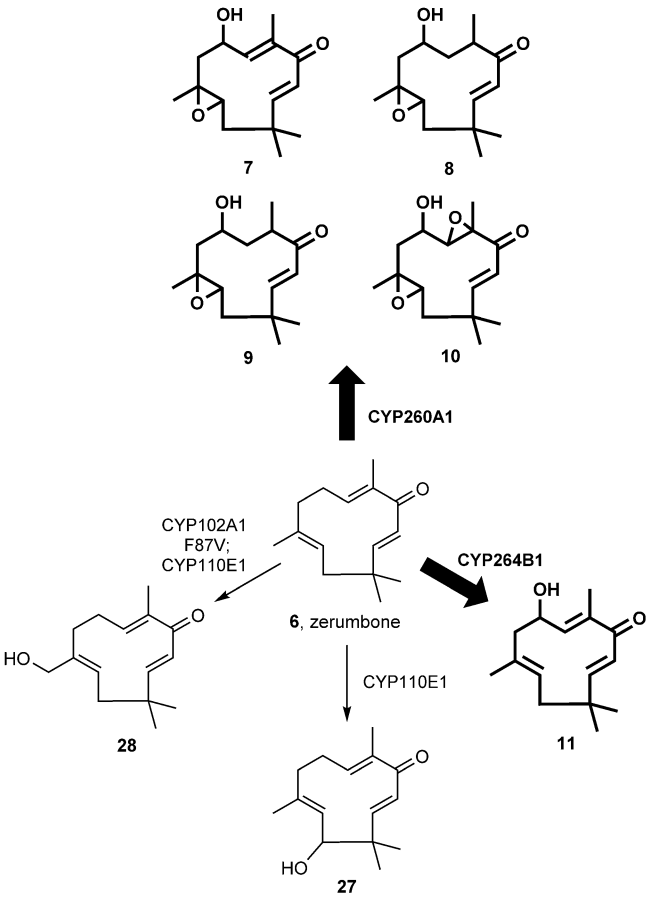
Filling gaps in the range of sesquiterpene oxidations

Most of the bacterial P450s that have been functionally investigated and found to oxidize sesquiterpenes are contained in biosynthetic clusters of different species,[33–38] including *S. cellulosum* So ce56, close to TPS genes.[27] Sesquiterpene-oxidizing P450s that are not clustered with TPS genes include CYP109B1, which performs the commercially attractive conversion of **4** into **1**,[39] CYP102A1 ($P450_{BM3}$) and CYP154E1, which both oxidize **1** to form **3**, **25**, and **26** (Scheme 4), and the cyanobacterial CYP110 family.[40] CYP110E1 hydroxylates **6** to various products, although yielding mainly **27** and **28** (Scheme 5).[41]

Compounds **1** and **6** are the most widely studied sesquiterpenes in terms of bacterial P450-mediated selective oxidations. The existing choice of tools, however, covers only a few oxidation positions of model substrates **1** and **6**. In this study we provide a new product—(4*S*)-hydroxynootkatone (**2**)—through hydroxylation of the chemically inert C-4 of **1**. We also provide another way to access the primary (+)-nootkatone alcohol **3**. Previous studies have shown only allylic hydroxylation products **3** and **26** and the epoxide **25** (Scheme 4).[42–44] Oxidation of **6** leads even to a greater variety of described products. Here, we contribute a new selective hydroxylation by CYP264B1 to yield 5-hydroxyzerumbone (**11**) and the multiply oxidized CYP260A1 products **7**–**10** (Scheme 5). The side products of the CYP260A1- and CYP264B1-catalyzed reactions include new and unidentified structures. Further development



Scheme 4. Oxidation products of (+)-nootkatone (**1**) obtained through conversion with different bacterial P450s. The products identified in this study and the enzymes used are shown in bold. The other derivatization studies of **1** to **3**, **25**, and **26** were conducted by Sowden et al.,[42] by Kolev et al.,[43] and by von Bühler et al.[44]



Scheme 5. Oxidation products of zerumbone (**6**) obtained through treatment with different bacterial P450s. The products identified in this study and the enzymes used are shown in bold. The derivatization of **6** by treatment with CYP110E1 and CYP102A1F87V was conducted by Makino et al.[41]

based on the previous successful engineering studies of CYP102A1,[41] CYP101,[42] and CYP106A2[32] and including the P450s that have been used in this study should lead to further changes in reaction selectivities, and consequently, to expansion of the range of sesquiterpene products.

Applications of the oxidized sesquiterpene products

Oxidized eremophilane- and humulane-type sesquiterpenes have been shown to exhibit various pharmaceutically interesting activities. Compounds **3** and **11** are inhibitors of NO production in interferon- and lipopolysaccharide-induced RAW264.7 macrophage cells.[45–46] Compound **3** and other oxidized derivatives of (+)-nootkatone (**1**) inhibit proliferation in human cancer cell lines A549 and HL-60.[47] The oxidized zerumbone (**6**) products **7**–**10** are structurally close to (9*E*)-humulene-2,3;6,7-diepoxide (< Scheme 3, **24**), a promising anti-acetylcholinesterase agent for the treatment of Alzheimer's disease.[28] Compounds **7**–**10** could thus be tested for similar functions. Generally, the introduction of functional groups by P450s paves the way for further derivatizations. Selectively oxidized sesquiterpenes might serve as lead structures for the development of compound libraries for screening for biological activities.

Another important field of application for oxidized sesquiterpenes is that of flavors and fragrances. Introducing an alcohol or keto function significantly alters the olfactory properties of natural terpene hydrocarbons and artificial sesquiterpene-like ketones.[48] Selectively oxidized sesquiterpenes could thus serve for structure–odor relationship studies.[49]

Conclusion

The increasing industrial demand for oxidized sesquiterpenoids as pharmaceuticals, flavors, and fragrances requires synthetic methods for selective oxidation of their hydrocarbon precursors.[39,50] The methods of organic synthesis are often not able to meet this need, due to low efficiencies and selectivities. Cytochromes P450 from plants are known as native terpene oxidases.[5,13] However, bacterial P450s have been little studied for sesquiterpene derivatization until now. In this study we have focused on conversions of a structural variety of sesquiterpenes by the two P450s CYP260A1 and CYP264B1 from *S. cellulosum* So ce56. Three structural sesquiterpene types—eremophilanes, humulanes, and cedranes (Scheme 1)—were converted by both of the enzymes. The humulane-related caryophyllene (Scheme 2, **14**) was selectively hydroxylated by CYP264B1. Using the model substrates (+)-nootkatone (**1**) and zerumbone (**6**), we were able to produce the new oxidized derivatives **2** and **7**–**11**, complementary to products formed by other P450s (Schemes 4 and 5).

We also observed side products in the conversion analyses (Scheme 2), but these were not produced in sufficient amounts for structural analysis by NMR spectroscopy. The activity of CYP264B1 might be improved and the selectivities of both CYP260A1 and CYP264B1 increased by engineering through different mutagenesis techniques.[42–43,51] In this way, new oxidation positions could be accessible, to build up a toolbox for selective sesquiterpene derivatization with the aid of P450s. P450 expression can be efficiently coupled with the MVA pathway and TPS genes in terpene-producing strains to test CYP260A1 and CYP264B1 for oxidations of commercially unavailable sesquiterpenes.[15,52] Cadinane- and cubebane-type sesquiterpenes, which have recently been discovered as products of a *S. cellulosum* So ce56 TPS,[53] represent potential new substrates for CYP260A1 and CYP264B1.

Overall, CYP260A1 and CYP264B1 have been shown to be versatile oxidases, able to convert various structural sesquiterpene classes. Therefore, both P450s are promising oxidases for other sesquiterpene types. CYP264B1 is a highly selective allylic hydroxylase, derivatizing oxidized and non-oxidized substrates. CYP260A1 exclusively converts oxy-sesquiterpenes, producing unselective product mixtures. It is, however, valuable for its ability to hydroxylate chemically inert carbon atoms (compound **1** at C-4, compound **16** at C-9), and could be engineered to achieve higher selectivities.

The oxidized sesquiterpenes obtained in this study have been shown to exhibit antiproliferative (compound **3**)[47] and NO-synthesis-inhibiting (compounds **3** and **11**) abilities.[45–46] The products **7–10** are structurally close to the acetylcholinesterase-inhibiting agent **24**[28] and, therefore, likely to show similar properties. The oxidized sesquiterpenes can serve as precursors for compound libraries for the improvement of these pharmaceutically interesting properties. Because oxidation can change the odor of a fragrance compound,[48] the obtained products should be tested for their qualities as flavors and fragrances, and they could be used for structure–odor relationship studies.

Experimental Section

Enzymes and chemicals: The substrate α-cedrene was provided by Dr. Fanny Lambert (V. Mane & Fils, Bar-Sur-Loup, France), and β-caryophyllene was provided by Prof. Dr. Danièle Werck-Reichhart (University of Strasbourg, France). Isopropyl β-D-1-thiogalactopyranoside (IPTG) and 5-aminolevulinic acid were purchased from Carbolution chemicals (Saarbrücken, Germany). Bacterial media were purchased from Becton Dickinson (Heidelberg, Germany). All other chemicals were obtained from standard sources in the highest purity available.

Strains: The *E. coli* strain BL21-Gold(DE3) for the whole-cell conversions was purchased from Agilent Technologies, and the *E. coli* strain C43(DE3) was purchased from Lucigen Corp. (Middleton, USA).

Expression vectors: The redox partners Fpr and Adx_{4-108} were used for the *E. coli*-based whole-cell conversions. CYP260A1 (pET17b) was applied with the duet vector (pCDF dFA) coding for Fpr and Adx_{4-108},[31] whereas a tricistronic vector (pETC4oFA) coding for CYP264B1, Fpr and Adx_{4-108} was used for the CYP264B1-dependent conversions.[27]

Media: Terrific broth medium consisted of (per liter H_2O) yeast extract (24 g), peptone (12 g), glycerol (4 mL), K_2HPO_4 (2.31 g), and KH_2PO_4 (12.54 g).

M9CA medium consisted of (per liter H_2O) Na_2HPO_4 (6 g), KH_2PO_4 (3 g), NaCl (0.5 g), NH_4Cl (1 g), casamino acids (4 g), glucose (4 g), $CaCl_2$ (1 M, 50 μL), $MgSO_4$ (1 M, 2 mL), and trace elements solution [2 mL, trace elements solution contained (per 50 mL H_2O) EDTA (2.5 g), $FeSO_4$ (250 mg), $ZnCl_2$ (25 mg), and $CuSO_4$ (5 mg)].

***E. coli*-based whole-cell conversions with CYP260A1**: The experiments were performed with *E. coli* BL21(DE3) gold cells. The cells were transformed with two plasmids, one for CYP260A1 (pET17b) and the other for the redox partners Fpr and Adx_{4-108} (pCDF dFA). The overnight culture was prepared in nutrient broth containing ampicillin (100 μg mL^{-1}) and streptomycin (50 μg mL^{-1}). The main culture with M9CA medium was inoculated with the overnight culture (dilution 1:100) and incubated at 37 °C in sealed baffled flasks. The induction of the corresponding genes was initiated by adding IPTG (1 mM) and 5-aminolevulinic acid (0.5 mM) when the optical density reached ≈0.9, and the culture was grown further at 28 °C. After 21 h of expression, the temperature was set to 30 °C, and the substrates (50 mM stock solution in EtOH) were added to a final concentration of 200 μM. The reaction was carried out for 48 h under the same conditions, quenched by addition of the same volume of ethyl acetate, and extracted twice. The organic phases were collected, pooled, and concentrated to dryness. The obtained extracts were stored at −20 °C until purification.

***E. coli*-based whole-cell conversions with CYP264B1**: *E. coli* C43(DE3) cells were transformed with the tricistronic vector pET-C4oFA and grown overnight in nutrient broth containing ampicillin (100 μg mL^{-1}) at 37 °C. Terrific Broth medium containing ampicillin (100 μg mL^{-1}) was inoculated with the overnight culture (dilution 1:100) and grown under the same conditions until the optical density reached ≈0.9. The expression was induced with isopropyl β-D-1-thiogalactopyranoside (1 mM) and 5-aminolevulinic acid (0.5 mM). After shaking for 24 h at 30 °C, the cells were harvested by centrifugation (4000 *g*) and resuspended in potassium phosphate buffer (50 mM, pH 7.4) containing glycerol (4%). The substrates (200 μM), IPTG (1 mM), and 5 aminolevulinic acid (0.5 mM) were added, and the conversion was carried out at 30 °C for a further 24 h in sealed baffled flasks. The reactions were stopped by cooling at 4 °C for 30 min, and then the same volume of ethyl acetate was added. The products were extracted twice, and the organic phases were pooled and concentrated to dryness. The extracts were kept at −20 °C until purification.

Purification of the products: The extracts of α-cedrene, β-caryophyllene, (+)-3(15)-cedren-4-ol, α-humulene, and (+)-valencene zerumbone (CYP260A1-dependent conversion) were dissolved in ethyl acetate and loaded on a silica gel column. Mixtures of ethyl acetate and hexane were used as mobile phase. The ratios of these solvents are substrate-dependent: β-caryophyllene (ethyl acetate/hexane 1:9), α-cedrene (1:99), (+)-3(15)-cedren-4-ol (8:2), α-humulene (1:9), (+)-valencene (5:95), and zerumbone (8:2 for the CYP260A1-dependent conversion). The collected fractions were analyzed by thin-layer chromatography (silica gel on PET) with the same solvent mixture as the mobile phase. The separated compounds were stained with *p*-anisaldehyde for detection.[31] The obtained products were pooled, concentrated to dryness, and prepared for the analysis by GC/MS or NMR spectroscopy.

The extracts of (+)-nootkatone (CYP260A1-dependent conversion) and zerumbone (CYP264B1-dependent conversion) were purified by preparative HPLC. A system consisting of a PU-2080 HPLC pump, an AS-2059-SF autosampler, and a MD-2010 multi-wavelength detector (Jasco, Gross-Umstadt, Germany) was used. The products of (+)-nootkatone were purified with a reversed-phase Nucleodur 100–5 C18 column (250×5 mm, Macherey–Nagel, Düren, Germany) and use of A) water containing acetonitrile (10%), and B) pure acetonitrile as mobile phase. A gradient from 30% to 90% B was used for the separation of the corresponding products. The product of CYP264B1-dependent zerumbone conversions were purified with an unmodified VP Nucleodur 100–5 column (250×10 mm, Macherey–Nagel) and use of hexane and ethyl acetate as mobile phase. The zerumbone product was separated under isocratic conditions (ethyl acetate/hexane 2:8). The product-containing fractions were pooled, concentrated to dryness, and prepared for further analysis by GC/MS or NMR spectroscopy.

GC/MS analysis of the products: GC/MS analysis was performed with a system consisting of an AI/AS 3000 autosampler, a DSQII quadrupole, a Focus GC column oven (Thermo Scientific), and a HP-5 ms capillary column (25 m×0.32 mm i.d., 0.52 μm film, Agilent Technologies). The compounds were analyzed under the following conditions: inlet pressure 50 kPa, column flow 10 mL min^{-1}, injection volume 1 μL, transfer line 280 °C, electron energy 70.5 eV, splitless mode. The starting oven temperature was 50 °C for 1 min; then the temperature was increased by 10 °C min^{-1} to 310 °C and held for 5 min. The carrier gas was He with a flow of 1 mL min^{-1}. Mass spectra were recorded in a *m/z* range of 20–350.

HPLC analysis of the (+)-nootkatone conversion by CYP260A1: HPLC analysis was performed with a system consisting of a PU-2080 HPLC pump, an AS-2059-SF autosampler, and a MD-2010 multi-wavelength detector (Jasco). A Nucleodur 100–5 C_{18} column (125×4 mm, Macherey–Nagel) was used at 40 °C. The mobile phase consisted of A) a mixture of water and acetonitrile (90:10), and B) pure acetonitrile. The samples were dissolved in a mixture of water and acetonitrile (70:30) and separated by use of the following gradient with a flow rate of 0.8 mL min^{-1}: 30% solvent B for 1 min, linear increase to 90% for 8 min, maintained for 1 min, immediate decrease to 30%, maintained for 5 min. (+)-Nootkatone and its oxidation products were detected at 254 nm.

NMR analysis and data for the obtained products: NMR spectra were recorded in $CDCl_3$ with a Bruker DRX 500 spectrometer (Institut für Pharmazeutische Biologie, Universität des Saarlandes). The 2D NMR spectra were recorded as gs-HH-COSY, gs-HSQC, and gs-HMBC. All chemical shifts are relative to $CHCl_3$ or $CDCl_3$ (δ = 77.00 ppm for ^{13}C NMR, δ = 7.24 ppm for ^{1}H NMR) with use of the standard δ notion.

(4*S*)-Hydroxynootkatone (2): ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.24 (s, 3H; H-14), 1.26 (s, 3H; H-15), 1.36 (m, 1H; H-8a), 1.62 (ddd, *J* = 13.0, 3.0 and 3.0 Hz, 1H; H-6a), 1.73 (s, 3H; H-13), 1.85 (m, 1H; H-8b), 1.96 (dd, *J* = 13.0, 13.0 Hz, 1H; H-6b), 2.31 (dddd, *J* = 15.5, 13.0, 3.0, 3.0 Hz, 1H; H-7), 2.42 (dd, *J* = 17.0, 1.0 Hz, 1H; H-3a), 2.43 (m, 1H; H-9a), 2.51 (m, 1H; H-9b), 2.76 (d, *J* = 17 Hz, 1H; H-3b), 4.73 (s, 2H; H-12), 5.83 ppm (br s, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 20.8 (C-13), 22.4 (C-14), 23.4 (C-15), 30.8 (C-8), 33.1, (C-9), 35.9 (C-6), 40.4 (C-7), 44.5 (C-5), 49.0 (C-3), 75.9 (C-4), 109.4 (C-12), 124.5 (C-1), 149.1 (C-11), 167.0 (C-10), 197.3 ppm (C-2).

13-Hydroxynootkatone (3): The NMR data for this compound are presented in Ly et al.[30]

13-Hydroxyvalencene (5): ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.85 (d, *J* = 6.4 Hz, 3H; H-15), 0.93 (s, 3H; H-14), 0.97 (d, *J* = 12.8 Hz, 1H; H-6a), 1.19 (m, 1H; H-8a), 1.40 (m, 3H; H-3, H-4), 1.80 (m, 1H; H-8b), 1.89 (m, 1H; H-6b), 1.92 (m, 1H; H-2a), 2.05 (m, 1H; H-9a), 2.08 (m, 1H; H-2b), 2.29 (m, 2H; H-7, H-9b), 4.11 (d, *J* = 6.1 Hz, 2H; H-13), 4.86 (s, 1H; H-12a), 5.00 (s, 1H; H-12b), 5.32 ppm (m, 1H; H-1); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 15.6 (C-15), 18.5 (C-14), 25.9 (C-2),

27.1 (C-3), 32.7, (C-9), 33.6 (C-8), 36.7 (C-7), 37.9 (C-5), 40.9 (C-4), 45.4 (C-6), 65.3 (C-13), 107.9 (C-12), 120.4 (C-1), 142.7 (C-10), 154.1 ppm (C-11).

2,3-Epoxy-5-hydroxyzerumbone (7): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.06$ (s, 3H; H-12, H-13), 1.28 (s, 3H; H-12, H-13), 1.21 (s, 3H; H-14), 1.29 (m, 1H; H-4a), 1.42 (m, 1H; H-1a), 1.91 (m, 1H; H-1b), 1.97 (d, $J = 1.5$ Hz, 3H; H-15), 2.63 (m, 1H; H-4b), 2.74 (dd, $J = 11.2$, 1.7 Hz, 1H; H-2), 4.58 (m, 1H; H-5), 5.92 ppm (d, $J = 9.7$, 1.5 Hz, 1H; H-6), H-9 and H-10 were not present; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 12.6$ (C-15), 16.8 (C-14), 24.0 and 29.7 (C-12 and C-13), 35.9 (C-11), 42.6 (C-1), 47.6 (C-4), 59.3 (C-3), 62.7 (C-2), 64.9 (C-5), 128.7 (C-9), 142.5 (C-7), 144.4 (C-6), 161.3 (C-10), 202.7 ppm (C-8).

6,7-Dihydro-2,3-epoxy-5-hydroxyzerumbone (8): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.13$ (s, 3H; H-12, H-13), 1.24 (s, 3H; H-12, H-13), 1.18 (s, 3H; H-14), 1.18 (d, $J = 6.8$ Hz, 3H; H-15), 1.33 (m, 2H; H-4a, H-1a), 1.89 (m, 2H; H-6a, H-1b), 2.08 (d, $J = 13.3$ Hz, 1H; H-4b), 2.16 (dd, $J = 15.7$, 3.3 Hz, 1H; H-6b), 2.63 (m, 1H; H-2), 2.79 (m, 1H; H-7), 3.33 (dd, $J = 10.2$, 6.9 Hz, 1H; H-5), 6.25 (d, $J = 16.0$ Hz, 1H; H-9), 6.29 ppm (d, $J = 16.0$ Hz, 1H; H-10); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 12.6$ (C-15), 17.5 (C-14), 23.4 and 29.6 (C-12 and C-13), 36.1 (C-11), 40.4 (C-1), 42.7 (C-6), 44.7 (C-7), 51.1 (C-4), 59.1 (C-3), 60.5 (C-2), 66.3 (C-5), 128.0 (C-9), 150.4 (C-10), 204.0 ppm (C-8).

6,7-Dihydro-2,3-epoxy-5-hydroxyzerumbone (9): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.13$ (s, 3H; H-12, H-13), 1.25 (s, 3H; H-12, H-13), 1.21 (d, $J = 7.0$ Hz, 3H; H-15), 1.22 (s, 3H; H-14), 1.31 (m, 1H; H-4a), 1.34 (m, 1H; H-1a), 1.70 (m, 1H; H-6a), 1.80 (dd, $J = 15.1$, 11.7 Hz, 1H; H-6b), 1.86 (m, 1H; H-1b), 2.13 (d, $J = 13.5$ Hz, 1H; H-4b), 2.61 (m, 1H; H-2), 2.63 (m, 1H; H-7), 3.32 (m, 1H; H-5), 6.25 (d, $J = 16.1$ Hz, 1H; H-10), 6.33 ppm (d, $J = 16.1$ Hz, 1H; H-9); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 16.7$ (C-15), 17.5 (C-14), 23.5 and 29.7 (C-12 and C-13), 36.3 (C-11), 39.8 (C-1), 45.4 (C-6), 46.4 (C-7), 50.2 (C-4), 59.3 (C-3), 60.1 (C-2), 68.1 (C-5), 124.9 (C-9), 151.2 (C-10), 206.5 ppm (C-8).

2,3:6,7-Diepoxy-5-hydroxyzerumbone (10): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.15$ (s, 3H; H-12, H-13), 1.25 (s, 3H; H-12, H-13), 1.37 (s, 3H; H-14), 1.37 (m, 1H; H-4b), 1.51 (dd, $J = 14.3$, 11.2 Hz, 1H; H-1a), 1.69 (s, 3H; H-15), 1.97 (m, 1H; H-1b), 2.60 (m, 1H; H-4b), 2.65 (dd, $J = 11.3$, 1.5 Hz, 1H; H-2), 2.78 (d, $J = 9.0$ Hz, 1H; H-6), 3.74 (m, 1H; H-5), 5.95 (d, $J = 17.2$ Hz, 1H; H-9), 6.83 ppm (d, $J = 17.2$ Hz, 1H; H-10); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 16.6$ (C-15), 17.6 (C-14), 25.1 and 30.1 (C-12 and C-13), 35.3 (C-11), 41.6 (C-1), 44.7 (C-4), 58.1 (C-3), 61.7 (C-2), 65.2 (C-5), 65.9 (C-6), 66.7 (C-7), 126.4 (C-9), 160.0 (C-10), 197.2 ppm (C-8).

5-Hydroxyzerumbone (11): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.06$ (s, 3H; H-12, H-13), 1.18 (s, 3H; H-12, H-13), 1.54 (s, 3H; H-14), 1.90 (s, 3H; H-15), 1.90 (m, 1H; H-1a), 2.13 (m, 1H; H-4a), 2.34 (t, $J = 12.8$ Hz, 1H; H-1b), 2.74 (m, 1H; H-4b), 4.62 (m, 1H; H-5), 5.28 (brd, $J = 10.9$ Hz, 1H; H-2), 5.79 (d, $J = 9.8$ Hz, 1H; H-6), 5.88 (d, $J = 16.5$ Hz, 1H; H-10), 5.98 ppm (d, $J = 16.5$ Hz, 1H; H-9); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 12.2$ (C-15), 16.5 (C-14), 24.2 and 29.3 (C-12 and C-13), 38.5 (C-11), 42.5 (C-1), 49.3 (C-4), 64.9 (C-5), 126.7 (C-2), 127.3 (C-9), 133.0 (C-3), 140.6 (C-7), 145.6 (C-6), 162.4 (C-10), 204.2 ppm (C-8).

5-Hydroxyhumulene (13): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.02$ (s, 3H; H-12, H-13), 1.07 (s, 3H; H-12, H-13), 1.45 (s, 3H; H-14), 1.70 (s, 3H; H-14), 1.80 (dd, $J = 13.5$, 5.2 Hz, 1H; H-1a), 1.98 (dd, $J = 13.5$,d 10.0 Hz, 1H; H-1b), 2.05 (dd, $J = 12.0$, 8.2 Hz, 1H; H-4a), 2.31 (m, 1H; H-8a), 2.46 (dd, $J = 12.0$, 5.2 Hz, 1H; H-4b), 2.71 (dd, $J = 13.8$, 8.3 Hz, 1H; H-8b), 4.54 (m, 1H; H-5), 4.94 (dd, $J = 9.7$, 5.3 Hz, 1H; H-2), 5.00, dt, $J = 9.6$, 1.4 Hz, 1H; H-6), 5.14 (d, $J = 16.0$ Hz, 1H; H-10), 5.58 ppm (m, 1H; H-9); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 16.7$ (C-14), 19.1 (C-15), 25.5 and 28.7 (C-12 and C-13), 36.9 (C-11), 39.4 (C-8), 41.7 (C-1), 49.3 (C-4), 67.0 (C-5), 126.5 (C-2), 127.5 (C-9), 130.0 (C-6), 131.7 (C-3), 141.5 (C-10), 141.9 ppm (C-7).

5-Hydroxycaryophyllene (15): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.94$ (s, 3H; H-12, H-13), 0.95 (s, 3H; H-12, H-13), 1.41 (t, $J = 9.7$ Hz, 1H; H-10), 1.53–1.58 (m, 3H; H-1a, H-8a, H-9a), 1.60 (s, 3H; H-14), 1.67 (m, 1H; H-9b), 1.78 (m, 1H; H-1b), 1.92 (t, $J = 10.6$ Hz, 1H; H-4a), 2.25 (dd, $J = 9.7$, 8.9 Hz, 1H; H-2), 2.51 (dd, $J = 13.7$, 6.7 Hz, 1H; H-8), 2.76 (dd, $J = 11.5$, 6.6 Hz, 1H; H-4b), 4.58 (m, 1H; H-5), 4.89 (s, 1H; H-15a), 4.98 (s, 1H; H-15b), 5.24 ppm (d, $J = 10.2$ Hz, 1H; H-6); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 21.8$ and 22.9 (C-12 and C-13), 29.8 (C-14), 31.2 (C-9), 32.7 (C-11), 34.7 (C-8), 42.2 (C-1), 48.9 (C-2), 49.2 (C-4), 55.5 (C-10), 70.8 (C-5), 112.4 (C-15), 127.8 (C-6), 136.9 (C-7), 150.0 ppm (C-3).

4,9-Dihydroxy-3(15)-cedrene (17): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.92$ (d, $J = 7.1$ Hz, 3H; H-14), 1.00 (s, 3H; H-12, H-13), 1.11 (s, 3H; H-12, H-13), 1.18 (dd, $J = 11.9$, 10.2 Hz, 1H; H-5a), 1.31 (d, $J = 11.6$ Hz, 1H; H-1a), 1.50 (m, 1H; H-8a), 1.65 (d, $J = 8.3$ Hz, 1H; H-10), 1.72 (m, 1H; H-1b), 1.78 (m, 1H; H-7), 2.08 (m, 1H; H-8b), 2.20 (m, 1H; H-5b), 2.35 (d, $J = 4.2$ Hz, 1H; H-2), 4.10 (m, 1H; H-9), 4.35 (m, 1H; H-4), 4.74 (t, $J = 2.1$ Hz, 1H; H-15a), 4.98 ppm (t, $J = 2.1$ Hz, 1H; H-15b); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 15.2$ (C-14), 26.4 and 26.5 (C-12 and C-13), 39.0 (C-7), 41.1 (C-11), 45.0 (C-5), 45.6 (C-1), 46.6 (C-8), 53.5 (C-6), 60.8 (C-2), 65.8 (C-10), 69.7 (C-4), 73.6 (C-9), 107.0 (C-15), 153.4 ppm (C-3).

3,15-Epoxy-4,9-dihydroxycedrane (18): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.93$ (d, $J = 7.1$ Hz, 3H; H-14), 1.10 (s, 3H; H-12, H-13), 1.17 (s, 3H; H-12, H-13), 1.22 (m, 1H; H-5a), 1.39 (m, 1H; H-2), 1.50 (AB q, $J = 2.0$ Hz, $\Delta\nu = 5.2$ Hz, 1H H-8a), 1.62 (m, 2H; H-1), 1.72 (d, $J = 8.2$ Hz, 1H; H-10), 1.80 (m, 1H; H-7), 2.08 (m, 1H; H-8b), 2.22 (m, 1H; H-5b), 2.61 (d, $J = 4.7$ Hz, 1H; H-15a), 3.05 (d, $J = 4.7$ Hz, 1H; H-15b), 4.10 (m, 1H; H-4), 4.11 ppm (m, 1H; H-9); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 15.1$ (C-14), 25.9 and 26.4 (C-12 and C-13), 39.1 (C-7), 40.3 (C-11), 42.3 (C-1), 43.2 (C-5), 46.5 (C-8), 51.7 (C-15), 52.9 (C-6), 58.1 (C-2), 61.5 (C-3), 65.1 (C-10), 65.3 (C-4), 73.5 ppm (C-9).

9-Hydroxy-4-keto-3(15)-cedrene (19): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.93$ (d, $J = 7.1$ Hz, 3H, H-14), 0.93 (s, 3H; H-12, H-13), 1.14 (s, 3H; H-12, H-13), 1.52 (AB q, $J = 2.2$ Hz, $\Delta\nu = 5.5$ Hz, 1H; H-8a), 1.59 (d, $J = 7.8$ Hz, 1H; H-10), 1.69 (d, $J = 12.2$ Hz, 1H; H-1a), 1.91 (m, 1H; H-7), 1.93 (m, 1H; H-1b), 2.11 (m, 1H; H-8b), 2.32 (dd, $J = 17.5$, 1.0 Hz, 1H; H-5a), 2.53 (dd, $J = 17.5$, 3.2 Hz, 1H; H-5b), 2.57 (d, $J = 4.2$ Hz, 1H; H-2), 4.15 (m, 1H; H-9), 5.04 (d, $J = 2.0$ Hz, 1H; H-15a), 5.90 ppm (d, $J = 2.0$ Hz, 1H; H-15b); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 15.4$ (C-14), 25.8 and 28.9 (C-12 and C-13), 39.3 (C-7), 42.2 (C-11), 42.8 (C-1), 46.3 (C-8), 51.6 (C-5), 53.1 (C-6), 58.3 (C-2), 67.9 (C-10), 73.5 (C-9), 121.1 (C-15), 148.2 (C-3), 201.1 ppm (C-4).

9-Hydroxy-4-ketocedrane (20): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.91$ (d, $J = 7.1$ Hz, 3H, H-14), 1.05 (s, 3H; H-12, H-13), 1.09 (s, 3H; H-12, H-13), 1.16 (d, $J = 7.0$ Hz, 3H, H-15), 1.45 (AB q, $J = 1.9$ Hz, $\Delta\nu = 5.1$ Hz, 1H; H-8a), 1.57 (d, $J = 8.4$ Hz, 1H; H-10), 1.78 (m, 1H; H-1a), 1.90 (m, 1H; H-7), 1.97 (m, 1H; H-2), 1.97 (m, 1H; H-1b), 2.10 (m, 1H; H-8b), 2.31 (d, $J = 14.5$ Hz, 1H; H-5a), 2.40 (dd, $J = 14.7$, 2.6 Hz, 1H; H-5b), 2.50 (m, 1H; H-3), 4.07 ppm (m, 1H; H-9). ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 14.5$ (C-15), 15.4 (C-14), 27.8 and 28.8 (C-12 and C-13), 38.5 (C-7), 43.5 (C-11), 46.5 (C-8), 47.1 (C-1), 51.7 (C-5), 51.8 (C-3), 55.3 (C-6), 56.7 (C-2), 67.7 (C-10), 73.4 (C-9), 212.5 ppm (C-4).

15-Hydroxy-3(4)-cedrene (22): ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.84$ (d, $J = 7.1$ Hz, 3H, H-14), 0.95 (s, 3H; H-12, H-13), 0.98 (s, 3H; H-12,

H-13), 1.34–1.38 (m, 3H; H-1a, H-8a, H-9a), 1.57 (m, 1H; H-9b), 1.66–1.78 (m, 3H; H-1b, H-2, H-7b), 1.82–1.87 (m, 2H; H-5a, H-8b), 1.93 (d, $J=3.8$ Hz, 1H; H-10), 2.23 (dd, $J=17.2$ and 1.4 Hz, 1H; H-7), 3.99 (AB q, $J=13.5$ Hz, $\Delta\nu=41.4$ Hz, 1H; H-15), 5.50 ppm (m, 1H; H-1); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=15.4$ (C-14), 24.8 (C-9), 25.5 (C-13), 27.7 (C-12), 36.1, (C-8), 38.6 (C-5), 40.6 (C-1), 41.4 (C-7), 48.4 (C-11), 50.4 (C-10), 54.3 (C-6), 59.1 (C-2), 67.2 (C-15), 120.4 (C-4), 144.1 ppm (C-3).

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