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Oxidation of acyclic monoterpenes by P450 BM-3 monooxygenase: influence of the substrate *E*/*Z*-isomerism on enzyme chemo- and regioselectivity

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Dedicated to Professor Rolf D. Schmid and Professor Jens Weitkamp on the occasion of their 65th birthday

Abstract—Oxidized terpenes and terpenoids are highly valuable compounds for organic chemistry. Cytochrome P450 monooxygenase P450 BM-3 from *Bacillus megaterium* is able to catalyze oxidation of terpenes with high efficiency. Mutations at the amino acid positions 47, 51, and 87 resulted in significantly enhanced activity and regioselectivity of the enzyme during oxidation of geranylacetone and related compounds. The activity of the mutant R47L/Y51F/F87V was in the order of ketone>alcohol>aldehyde>acid. An effect of the substrate cis/trans-isomerism on the enzyme chemo- and regioselectivity was studied. P450 monooxygenase demonstrated similar NADPH turnovers with cis/trans isomers, nerylacetone/geranylacetone ($1.9\times10^3/2.1\times10^3$ min^{-1}) and nerol/geraniol ($5.7\times10^2/5.9\times10^2$ min^{-1}), however, resulted in different number of products and product distribution. The *Z*-isomers, nerylacetone and nerol, were oxidized resulting in several products (five and three, respectively), including allylic alcohols. In contrast, *E*-isomers were epoxidized exclusively. Geranylacetone was converted with high activity (2080 min^{-1}) and enantioselectivity (97% ee) to 9,10-epoxygeranylacetone, while geraniol was enantioselectively epoxidized to the 6,7-epoxide (250 min^{-1}, 90% ee) with 90% regioselectivity.

1. Introduction

Cytochrome P450 monooxygenase from *Bacillus megaterium* P450 BM-3 (CYP102A1) is considered as one of the most promising monooxygenases for implementation in preparative synthesis. P450 BM-3 is a catalytically self-sufficient flavocytochrome containing a P450 heme domain and an NADPH-dependent diflavin reductase domain in one polypeptide chain.[1,2] This P450 monooxygenase is relatively stable under process conditions.[3] The turnover frequencies toward fatty acids are the highest among those reported for other P450 monooxygenases. Particular high activities are reported for unsaturated fatty acids (e.g., k_{cat}=230 s^{-1} for arachidonic acid).[4] Therefore they are often considered as natural substrates of P450 BM-3. Nevertheless, straight chain and branched fatty acids may also represent natural substrates for this monooxygenase, as they are oxidized with very high activities as well.[1,5] Besides fatty acids, a wide range of other compounds is known to be converted by the engineered P450 monooxygenase. P450 BM-3 mutants have been described, which are able to oxidize alkanes,[6] polycyclic aromatic hydrocarbons,[7] and human metabolites.[8–10] Among these compounds terpenes are particularly interesting substrates. Wong et al.[11] reported that some P450 BM-3 mutants converted monoterpene geraniol into more than four oxidized compounds including epoxides and alcohols. Mutants of cytochrome P450 monooxygenase from *Pseudomonas putida* (CYP101A1) were more chemo- and regioselective in this reaction and produced two compounds. Obviously, the regioselectivity of the monooxygenases is dependent on the structure of their binding sites. Nevertheless, the substrate configuration (e.g., stereochemistry) has a pronounced influence on the regioselectivity of P450 monooxygenases. For example, CYP118 has recently been described to yield four products from the oxidation of (+)-limonene and one single product in the reaction with (−)-limonene.[12] β-Ionone was regioselectively hydroxylated by some mutants of P450 BM-3 at position 4,[13] whereas its regioisomer α-ionone was oxidized at several positions[14]. Remarkably, α- and β-ionone produced only hydroxylated compounds, but no epoxides. The mechanisms

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of the regioselective hydroxylation of β-ionone in contrast to α-ionone by P450 BM-3 mutants and the preference of hydroxylation over epoxidation are yet unclear.

Our work was further motivated by the fact that several of the terpene epoxides are constituents of insect juvenile hormones, pheromones, and fragrances. For example, aromatic ethers of 6,7-epoxygeraniol have been identified as potent juvenile hormones in several beetles.[15,16] 2,3-Epoxygeraniol and the corresponding acetate are part of the pheromone bouquet of male neotrophical orchid bees,[17] whereas 2,3-epoxynerol is used by acarid mites as sex pheromone.[18] In addition, acyclic terpene-derived epoxides have been used as valuable building blocks, e.g., for the synthesis of the aggregation pheromone of the Colorado potato beetle,[19] α-damascone,[20,21] 2,2-dialkylchromanols[22] or antibiotic terpene (+)-tuberine,[23] diterpenoid peucelinendiol,[24] and decalins.[25]

Oxidation of several acyclic monoterpenes by P450 BM-3 and its mutants was thus investigated in this work to clarify the chemo- and regioselectivity of the monooxygenase.

2. Results and discussion

2.1. Effect of mutations on oxidation of geranylacetone

P450 BM-3 preferentially oxidizes subterminal carbon of fatty acids.[26,27] Its NADPH turnover toward saturated fatty acids is known to be several thousands per minute.[4] On the other hand, the activities toward other compounds are relatively low.[14,28] Actually, the NADPH turnover during oxidation of 6,10-dimethyl-5,9-undecadien-2-one (geranylacetone) **1** by wild type P450 BM-3 was 81 mol mol^{-1} P450 monooxygenase min^{-1} (further given as min^{-1}) (Table 1). Crude preparations of 25 mutants of P450 BM-3 stored in our laboratory were screened using an NADPH oxidation assay. These mutants contain single or combinations of up to five substitutions located along the substrate access channel and within the active center at following positions R47, Y51, A74, F87, and L188. The assay allowed a fast identification of active mutants with high NADPH turnovers, and therefore is a proper method for screening. Selected results are shown in Table 1. Triple mutant, A74G/F87V/L188Q demonstrated an 11-fold increased NADPH turnover compared to the wild type enzyme. This mutant has been described in numerous previous publications as active toward a broad range of substrates.[14] A 6-fold increase in activity toward geranylacetone was demonstrated by a mutant, which had amino acid substitutions of arginine 47 by leucine and tyrosine 51 by phenylalanine (R47L/Y51F). Arginine at position 47 and tyrosine at position 51 are believed to be involved in orientation of fatty acids in the substrate binding channel of P450 BM-3. Their substitutions resulted usually in decreased turnover rates with fatty acids,[27] but in an improved activity toward nonnatural hydrophobic bulky substrates, like polycyclic aromatic hydrocarbons.[7] Amino acid substitution of phenylalanine 87 by valine, described previously,[29] in addition to substitutions R47L and Y51F (R47L/Y51F/F87V) increased the turnover 26-fold. Introduction of mutation L188Q (replacement of leucine 188 by glutamine) to the triple mutant (R47L/Y51F/F87V/) did not lead to an increase in activity (18-fold compared to wild type).

Table 1. Relative NADPH turnover of P450 BM-3 mutants toward geranylacetone **1**

P450 BM-3	Relative NADPH turnover[a]
Wild type	1.0
R47L/Y51F	6.4
R47L/Y51F/F87V	26.0
R47L/Y51F/F87V/L188Q	18.0
A74G/F87V/L188Q	11.0

[a] NADPH turnovers of P450 BM-3 variants are given relative to that of wild type (1).

The NADPH oxidation assay, however, only shows the reductase activity of P450 BM-3 mutants. It does not provide any information about products formed, e.g., the oxygenase activity. Therefore, diethylether extracts of the reaction mixtures were analyzed by gas chromatography–mass spectroscopy (GC–MS, Table 2). When 90% of the substrate was converted by wild type P450 BM-3, three peaks appeared in the ratio of 1:60:39. In contrast, one compound (peak 2) was mainly detected in the ether extracts of the reaction mixtures of the two triple mutants, R47L/Y51F/F87V and A74G/F87V/L188Q. These results indicated that the mutations in R47L/Y51F/F87V and A74G/F87V/L188Q increased not only NADPH oxidation rate but also the chemo- and regioselectivity of P450 BM-3 toward geranylacetone **1**. As the triple mutant R47L/Y51F/F87V had the highest NADPH turnover, it was chosen for further investigations.

Table 2. Oxidation of geranylacetone **1** by P450 BM-3 mutants

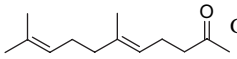
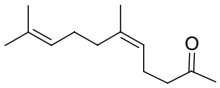
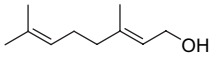
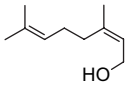
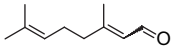
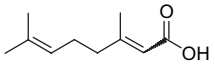
P450 BM-3	Enzyme amount (μM)	Substrate conversion[a] (%)	Product distribution[a] (ratio of peak area, %)		
			Peak 1[a]	Peak 2[a]	Peak 3[a]
Wild type	0.40	90	1	60	39
R47L/Y51F/F87V	0.025	89	n.d.	100	n.d.
A74G/F87V/L188Q	0.04	90	2	98	n.d.

n.d.—not detected.
[a] Substrate conversion and product distribution were calculated based on the peak area ratio between geranylacetone **1** and the internal standard, nerol **4**, before and after reactions.

2.2. Substrate specificity of R47L/Y51F/F87V

The mutant R47L/Y51F/F87V monooxygenase was purified by the metal affinity chromatography as described in Section 4 and tested with geranylacetone **1** and structurally related compounds **2**–**6** (Table 3). Geranylacetone **1** was the best substrate, causing the highest NADPH turnover of 2.1×10^3 min^{-1}, followed by its *Z*-isomer, nerylacetone **2** with turnover of 1.9×10^3 min^{-1}. With geraniol **3** and its *Z*-isomer, nerol **4**, R47L/Y51F/F87V showed NADPH turnovers of 590 and 570, respectively. This result indicated that *Z*- and *E*-configuration of the substrate did not significantly affect the NADPH turnover rates. When a mixture of *Z*- and *E*-isomer of citral **5** (*Z*/*E*=38:62) was used, NADPH turnover decreased to 270 min^{-1}. Geranic acid **6** was the worst substrate for R47L/Y51F/F87V, showing the lowest turnover of 23 min^{-1}. Unnatural substrates that bind to an

Table 3. Reaction of R47L/Y51F/F87V-P450 BM-3 with geranylacetone **1** and structurally related terpenes **2–6**

Substrate	R47L/Y51F/F87V			A74G/F87V/L188Q		
	NADPH turnover (min^{-1})	Coupling efficiency (%)	Activity[a] (min^{-1})	NADPH turnover (min^{-1})	Coupling efficiency (%)	Activity[a] (min^{-1})
 Geranylacetone **1**	2100	99	2079	1380	99	1366
 Nerylacetone **2**	1900	91	1700	1300	91	1183
 Geraniol **3**	590	42	250	440	81	356
 Nerol **4**	570	46	260	645	97	626
 Citral **5**[b]	270	77	210	200	91	182
 Geranic acid **6**[b]	23	n.d.	n.d.	40	n.d.	n.d.

n.d.—not determined.
[a] Activity is the value obtained by multiplying NADPH turnover and the coupling efficiency.
[b] Substate was a (38:62) mixture of *Z*- and *E*-isomer.

enzyme in an unfavorable orientation can often lead to a so-called uncoupling between NADPH consumption and product formation. Usually the yield of product based on NAD(P)H consumption is defined as coupling efficiency. The coupling efficiency is thus an important criterion to evaluate the monooxygenase activity.

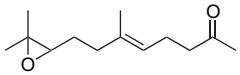
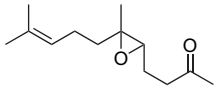
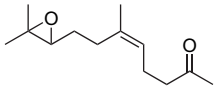
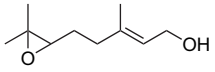
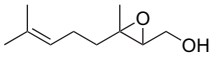
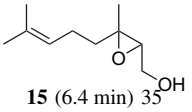
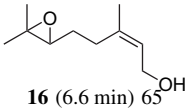
However, all of the oxidized products could not be quantified here because of the lack of the authentic compounds (see below). The coupling efficiency was, thus, calculated as the proportion of the substrate converted to NADPH oxidized (Table 3). Then, the monooxygenase activity was indicated by the multiplied value of NADPH turnover and the coupling efficiency. R47L/Y51F/F87V showed the highest activities of 2100 min^{-1} and 1700 min^{-1} toward geranylacetone and nerylacetone, respectively. The activities were surprisingly comparable to those of the wild type P450 BM-3 with fatty acids. The activities of R47L/Y51F/F87V with geraniol, nerol, and citral were 250 min^{-1}, 260 min^{-1}, and 210 min^{-1}, respectively. These results indicated that not only the NADPH turnover but also the comprehensive activity of R47L/Y51F/F87V toward these compounds is in the order of ketone >alcohol>aldehyde>acid, regardless of *Z*- and *E*-configuration of the substrate. This result differs from the specificity of wild type P450 BM-3 toward saturated fatty acids and alcohols, which has been reported previously. Fatty acids are hydroxylated 2 to 50 times faster than their corresponding alcohols.[30] To clarify if this unexpected order of reactivity is due to the altered amino acid residues at positions R47 and Y51, we tested P450 BM-3 wild type with the respective terpenes. The NADPH oxidation rate decreased from the ketones to the alcohols and aldehyde and then to the acid as it was observed for the R47L/Y51F/F87V triple mutant. However, the overall activity was low (<100 min^{-1}) and no products could be detected in reactions with geranic acid and citral. Therefore we carried out the same experiment using the A74G/F87V/L188Q triple mutant with the unaltered R47/Y51 motif. Also here the reactivity decreased from the ketones to the acid as was measured for wild type and the R47L/Y51F/F87V mutant. As a consequence, this order of reactivity cannot be linked to the nature of amino acids at positions 47 and 51 but to the nature of the substrates alone.

2.3. Products from geranylacetone and related compounds

Geranylacetone **1** and terpenes **2–6** were oxidized with R47L/Y51F/F87V and the substrate conversion and product distribution was analyzed by GC–MS and nuclear magnetic resonance (NMR) (Table 4). Ninety percent of geranylacetone **1** was converted enantioselectively to a single product (97% ee), which was identified as *E*-6,10-dimethyl-9,10-epoxy-5-undecen-2-one **7** (Fig. 1, A and B). The absolute configuration of stereogenic centers could not be determined due to the lack of enantiopure authentic samples. Conversion of nerylacetone **2**, the *Z*-isomer of geranylacetone **1**, was also very high and reached 88%. However, five different products appeared in a ratio of 39:8:24:21:8. All of them showed molecular ions at *m/z* 210, which is due to the introduction of oxygen into nerylacetone **2**. By comparing EIMS and NMR spectra with authentic samples, the two main products were determined as *Z*-6,10-dimethyl-5,6-epoxy-9-undecen-2-one **8**, and *Z*-6,10-dimethyl-9,10-epoxy-5-undecen-2-one **10**, respectively.

Oxidation of the alcohols, geraniol **3** and nerol **4**, led to two products in each case after 80% substrate conversion. Products of geraniol **3** were elucidated to be *E*-3,7-dimethyl-6,7-epoxy-2-octen-1-ol **13** and *E*-3,7-dimethyl-2,3-epoxy-6-octen-1-ol **14**, which were produced in a ratio of 90:10. Formation of *E*-3,7-dimethyl-6,7-epoxy-2-octen-1-ol **13**

Table 4. Oxidation products of geranylacetone **1** and its structurally related compounds **2–6** after reaction with R47L/Y51F/F87V-P450 BM-3

Substrate (conversion, %)	Product distribution: estimated structure[a] (retention time) area[b] (%)			
Geranylacetone **1** (90)	 **7** (11.1 min) 100			
Nerylacetone **2** (88)	 **8** (7.8 min) 39	**9** (9.7 min) 8	 **10** (10.1 min) 24	**11** (10.2 min) 21, **12** (11.1 min) 8
Geraniol **3** (81)	 **13** (7.0 min) 90	 **14** (7.4 min) 10		
Nerol **4** (80)	 **15** (6.4 min) 35	 **16** (6.6 min) 65		
E-Citral **5** (68)	**17** (6.4 min) trace	**18** (6.7 min) 20	**19** (6.8 min) 5	**20** (7.3 min) 75
Geranic acid **6** (24)	n.d.			

n.d.—not detected.
[a] Determined by NMR.
[b] Determined by GC.

was highly enantioselective (90% ee) (Fig. 1, C and D). Products of nerol **4** were determined as *Z*-3,7-dimethyl-2,3-epoxy-6-octen-1-ol **15** and *Z*-3,7-dimethyl-6,7-epoxy-2-octen-1-ol **16** in a ratio of 35:65.

These results suggested that R47L/Y51F/F87V preferentially catalyzed the epoxidation of the compounds **1–4**. It was also found that the regioselectivity of R47L/Y51F/F87V was higher toward *E*-isomers of the corresponding terpenes.

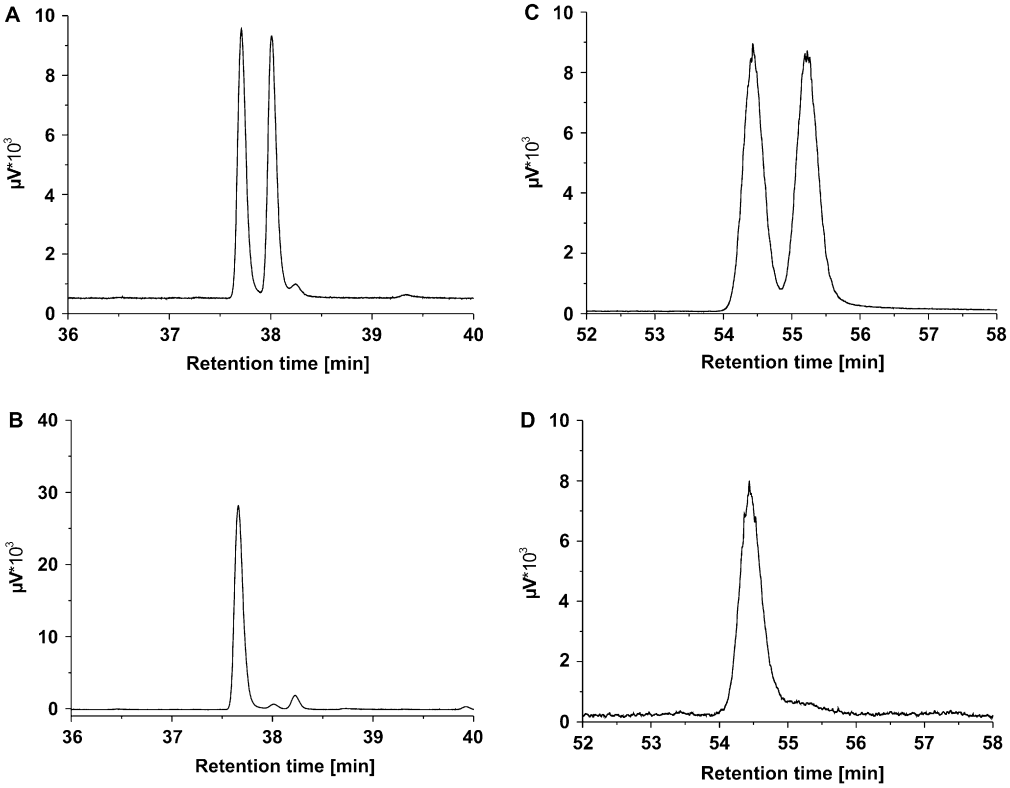


Figure 1. Gas chromatograms of enantiomer separation on a α-Cyclodex column. (A) Separation of racemic 9,10-epoxy-geranylacetone; (B) chromatogram of 9,10-epoxy-geranylacetone derived from reaction with P450 BM-3 R47L/Y51F/F87V (97% ee); (C) separation of racemic 6,7-epoxy-geraniol; (D) 6,7-epoxy-geraniol from enzymatic conversion of geraniol (90% ee).

From a mixture of *Z*- and *E*-citral **5** (*Z*/*E*=38:62), the monooxygenase produced four compounds **17**–**20** (Table 4). As given in Section 4, all of them had a molecular ion with *m*/*z* 168 by EIMS analysis, which corresponded with the transfer of one oxygen atom to citral **5**. The result might indicate that citral **5** was either epoxidized or hydroxylated by R47L/Y51F/F87V. In this case we were not able to obtain suitable reference compounds.

Although NADPH oxidation was detected when geranic acid **6** was used as substrate, no product was detected in GC–MS analysis. This may indicate that the coupling efficiency of R47L/Y51F/F87V was extremely low with geranic acid **6**.

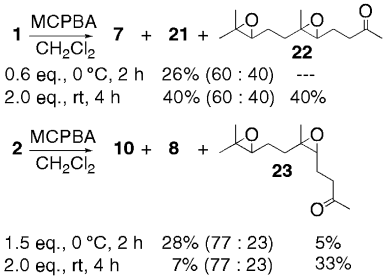
2.4. Chemical synthesis of terpene-derived epoxides

As mentioned above, the oxidation products of the monooxygenase-catalyzed reactions were analyzed by GC–MS (EI) and NMR spectroscopy. However, in order to allow an unambiguous determination of the products from the crude mixtures we decided to synthesize terpene-derived epoxides from **1**–**4** as reference compounds.

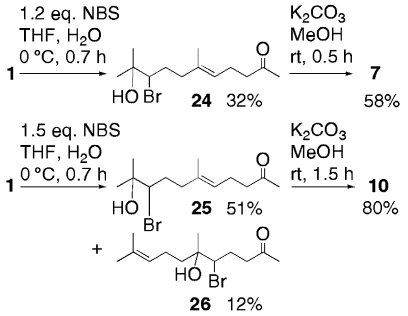
Following a modified procedure by Yue and Li[31] geranylacetone **1** was treated with 0.6 equiv of *m*-chloroperbenzoic acid (MCPBA) in dichloromethane at 0 °C to give an inseparable (60:40) mixture of 9,10-epoxide **7** and the corresponding 5,6-epoxide **21** in 26% yield (Scheme 1). When stoichiometric amounts of MCPBA were used, the undesired bisepoxide **22** was formed. The use of 2 equiv of oxidant gave 40% of **7** and **21** in a ratio of (60:40) together with 40% of the undesired bisepoxide **22**.

A similar phenomenon was observed for nerylacetone **2** (Scheme 1). With excess of MCPBA the bisepoxide **23** was the major product (33% yield) and the monoepoxides **10** and **8** were isolated as (77:23) a mixture in 7% yield. By using only 1.5 equiv of MCPBA the ratio of **10**, **8** versus **23** was just reversed.

In order to obtain *E*-6,10-dimethyl-9,10-epoxy-5-undecen-2-one **7** and *Z*-6,10-dimethyl-9,10-epoxy-5-undecen-2-one **10** in pure form, a different approach was tested (Scheme 2). Following a procedure by Zoretic et al.[32] geranylacetone **1** was treated with *N*-bromosuccinimide (NBS) in THF/water at 0 °C to give the bromohydrine **24** in 32%, which was subsequently reacted with K_2CO_3 in MeOH at room temperature to yield 58% of the desired 9,10-epoxide **7**. When nerylacetone **2** was submitted to NBS under similar conditions, the 9,10-bromohydrine **25** was isolated in 51% yield together with 12% of the 5,6-bromohydrine **26**. Compound **25** underwent clean nucleophilic displacement of the bromide to the corresponding epoxide **10** in 81% yield.

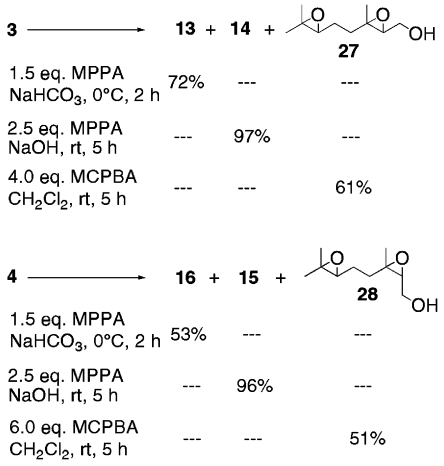


Scheme 1. Chemical epoxidation of geranylacetone with MCPBA.



Scheme 2. Chemical epoxidation of geranylacetone via a bromohydrine.

In contrast to geranylacetone **1** and nerylacetone **2** it turned out to be much easier to achieve regioselective epoxidation for geraniol **3** and nerol **4**. As shown in Scheme 3 geraniol **3** was treated with 1.5 equiv of monoperoxyphthalic acid (MPPA) in the presence of $NaHCO_3$ to give exclusively the 6,7-epoxide **13** in 72% yield following a procedure by Fringuelli et al.[33]



Scheme 3. Chemical epoxidation of geraniol with MPPA.

Under analogous conditions nerol **4** was converted to the 6,7-epoxide **16** in 53% yield. When 2.5 equiv of MPPA was used with **3** and **4** in the presence of NaOH at room temperature, the 2,3-epoxides **14** and **15** were obtained in 97% and 96% yield, respectively. The use of an excess of MCPBA and $NaHCO_3$ with **3** and **4** resulted in both cases in the formation of the bisepoxides **27** and **28** in 61% and 51% yield, respectively.

By having synthetic access to the terpene-derived bisepoxides **22**, **23**, **27**, and **28** we could unequivocally prove that monooxygenase-catalyzed reactions of terpenes **1**–**4** did not give any trace of the bisepoxides **22**, **23**, **27**, and **28**.

3. Conclusions

It was shown that the amino acid substitution at the position of 47, 51, and 87 of P450 BM-3 contributed to the enhanced

oxidation activity and regioselectivity toward geranylacetone **1** compared to wild type P450 BM-3. The activity of the mutant R47L/Y51F/F87V toward geranylacetone **1** and structurally related compounds **2–6** was in the order of ketone>alcohol>aldehyde>acid. *Z*/*E*-configuration did not significantly affect the NADPH turnover of the monooxygenase, whereas it strongly affected the product distribution. The oxidation of *Z*-isomers, nerylacetone **2** and nerol **4**, resulted in a mixture of several products, which were mainly epoxides. On the other hand, the *E*-isomers, geranylacetone **1** and geraniol **3**, were exclusively epoxidized at the positions of the C=C double bond located at C-9,C-10 (geranylacetone **1**) and at C-9,C-10 and C-6,C-7 (geraniol **3**) with outstanding enantioselectivity (97% ee for **7** and 90% ee for **13**). Comparison with reference compounds obtained by chemical epoxidation of terpenes **1–4** clearly revealed that the P450 BM-3 mutant did not produce any bisepoxides.

4. Experimental

4.1. Chemicals

Monoterpenes and other chemicals were purchased from Fluka (Buchs, Switzerland), Riedel-de Haën (Seelze, Germany) or Sigma (Deisenhofen, Germany). NADPH tetrasodium salt was a product of Jülich Fine Chemicals, Jülich, Germany. All chemicals were of analytical grade.

4.2. Preparation of P450 BM-3 monooxygenase and its mutants

Mutants R47L/Y51F, R47L/Y51F/F87V and A74G/F87V/L188Q were generated as described elsewhere.[3] In order to construct R47L/Y51F/F87V/L188Q, the 0.4 kb DNA-fragment was obtained by restricting R47L/Y51F/F87V cloned in pET28a(+) with *Bam*HI and *Mun*I endonucleases. By the identical procedure, 9.0 kb fragment was obtained from A74G/F87V/L188Q cloned in pET28a(+). The two corresponding fragments were then ligated by T4-DNA ligase by standard method.[34] The construct was introduced in *Escherichia coli* BL21(DE3) cells. Protein expression in *E. coli* and crude enzyme's preparation were carried out as described previously.[35] The purified R47L/Y51F/F87V was prepared by an affinity chromatography using the Hi-Trap Ni Sepharose FF column (5 ml, GE healthcare, Munich, Germany). The recovered monooxygenase fraction was dialyzed against 50 mM potassium–phosphate buffer (pH 7.5) to remove imidazole and NaCl. By the procedure, 69 nmol monooxygenase was obtained. The concentration of P450 BM-3 monooxygenases was determined from the CO-binding difference spectra of the reduced heme iron as reported by Omura and Sato,[36] using an extinction coefficient of 91 $mM^{-1}\,cm^{-1}$.

4.3. NADPH oxidation assay

The NADPH oxidation was monitored photometrically at the wavelength of 340 nm as described elsewhere.[14,37] The 1 ml reaction mixture contained 0.2 mM substrate, 0.2 mM NADPH, 2% DMSO, and 50 mM potassium–phosphate buffer (pH 7.5). Reaction was started by adding a given amount (0.02–0.12 μM) of P450 BM-3 preparation at 25 °C. The observed rates of NADPH oxidation were corrected for the slow background reaction, which was conducted under the identical condition without enzyme. NADPH turnover was calculated using the extinction coefficient, ε=6.22 $mM^{-1}\,cm^{-1}$. The coupling efficiency was calculated as the proportion of mole substrate converted measured by GC to mole NADPH consumed.

4.4. Reaction

The 1 ml reaction was carried out in 1.5 ml microtube preparation at 25 °C. The 50 ml reaction was carried out in a 100 ml round-bottle flask at 25 °C under stirring at 200 rpm. Reaction mixtures consisted of 0.2 mM substrate, 0.1–1.0 mM NADPH, 2% DMSO, 50 mM potassium–phosphate buffer (pH 7.5), and P450 BM-3 preparations. The amount of purified P450 BM-3 was as follows: 0.02 μM for geranylacetone **1** and nerylacetone **2**; 0.08 μM for geraniol **3** and nerol **4**; 0.2 μM for citral **5**; 2.0 μM for geranic acid **6**. A 50-ml scale reactions (for NMR analysis) were conducted for 24 h under same conditions except that NADPH concentration was fixed at 1.0 mM.

4.5. Product analyses

The reaction mixtures were extracted twice with diethylether. When terpenes except nerol **4** were used as substrates, 0.1 mM nerol **4** was used as an internal standard. When nerol **4** was used as substrate, 0.1 mM geraniol **3** was used as an internal standard.

Extracts were combined, evaporated to dryness, and dissolved in 0.1 ml of diethylether. GC–MS was performed on Shimadzu GC–MS QP2010 (Kyoto, Japan) equipped with an FS Supreme-5 column (0.25 mm×30 m, 0.25 μm, CS-Chromatographie Service, Langerwehe, Germany). The extracts, except from samples containing geranic acid **6**, were analyzed as follows: the column temperature was controlled at 120 °C for 2 min. The temperature was then raised to 200 °C at the rate of 5 °C min^{-1}, and then to 280 °C at 20 °C min^{-1}. It was kept at 280 °C for 1 min. The temperatures of the injector and the interface were fixed at 300 and 285 °C, respectively. As for samples containing geranic acid, the column temperature was controlled at 80 °C for 2 min. The temperature was then raised to 120 °C at the rate of 4 °C min^{-1}, and then to 280 °C at 20 °C min^{-1}. It was kept at 280 °C for 1 min.

Diethylether extracts of the reaction mixtures without internal standard were analyzed by EIMS and NMR. Mass spectra were collected using EI at 70 eV. NMR spectroscopic analyses were conducted in DMSO using a Bruker *Avance* NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany).

Separation of enantiomers of 6,7-epoxy-geraniol and 9,10-epoxy-geranylacetone was carried out by gas chromatography on a Shimadzu GC2010 (Kyoto, Japan) GC equipped with a FS-Cyclodex α-I/P column (0.25 mm×50 m; CS-Chromatographie Service, Langerwehe, Germany). The GC oven was kept isothermal at 120 °C for separation of 6,7-epoxy-geraniol enantiomers, analysis of 9,10-epoxy-

geranylcetone enantiomers was done using a linear temperature gradient of 2.5 °C min^{-1} from 40 to 200 °C.

4.6. Identification and elucidation of products

Only the data of the non-identified products are given. For all other oxidation products see below:

Compound **9**: EIMS 70 eV, *m/z* (rel int. %): 210 $[M]^+$ (6), 192 $[M-H_2O]^+$ (3), 134 (8), 128 (50), 126 (24), 110 (26), 109 (27), 108 (13), 101 (63), 96 (27), 85 (37), 83 (35), 71 (86), 69 (85), 55 (35), 43 (10).

Compound **11**: EIMS 70 eV, *m/z* (rel int. %): 210 $[M]^+$ (0.2), 192 $[M-H_2O]^+$ (0.2), 134 (3), 128 (21), 125 (10), 109 (12), 101 (19), 95 (19), 85 (23), 84 (43), 83 (31), 71 (16), 69 (26), 67 (18), 55 (25), 43 (100).

Compound **12**: EIMS 70 eV, *m/z* (rel int. %): 192 $[M-H_2O]^+$ (1), 149 (3), 135 (2), 134 (7), 126 (15), 119 (8), 111 (6), 109 (7), 108 (21), 107 (6), 93 (11), 85 (100), 83 (15), 71 (12), 69 (12), 55 (12), 43 (59).

Compound **17**: EIMS 70 eV, *m/z* (rel int. %): 168 $[M]^+$ (8), 150 $[M\text{-}H_2O]^+$ (1), 123 (14), 100 (26), 69 (100), 41 (34).

Compound **18**: EIMS 70 eV, *m/z* (rel int. %): 168 $[M]^+$ (0.4), 153 (0.5), 150 $[M-H_2O]^+$ (0.3), 139 (0.6), 125 (4), 110 (21), 95 (28), 82 (100), 59 (34), 41 (26).

Compound **19**: EIMS 70 eV, *m/z* (rel int. %): 168 $[M]^+$ (9), 153 (0.9), 150 $[M-H_2O]^+$ (0.8), 140 (0.4), 123 (17), 100 (23), 82, (5), 69 (100), 41 (37).

Compound **20**: EIMS 70 eV, *m/z* (rel int. %): 168 $[M]^+$ (0.3), 153 (1.5), 150 $[M-H_2O]^+$ (0.3), 139 (2.3), 135 (0.7), 125 (8), 97 (39), 81 (100), 59 (74), 41 (42).

4.6.1. Mixture of *E*-6,10-dimethyl-9,10-epoxy-5-undecen-2-one (7) and *E*-6,10-dimethyl-5,6-epoxy-9-undecen-2-one (21). To an ice bath cooled (0 °C) solution of geranylacetone **1** (200 mg, 1.03 mmol) in CH_2Cl_2 (6 ml) was added slowly MCPBA (77%, 134 mg, 0.600 mmol) and stirred at 0 °C for 2 h. The mixture was diluted with 1 N NaOH solution (5 ml) and the organic layer was washed with water (2×10 ml), dried (Mg_2SO_4), and concentrated. The residue was purified by flash chromatography on SiO_2 with EE/PE [1:10] to give products **7** and **21** (57 mg, 0.271 mmol, 26%) as colorless oil and as a (60:40) mixture. ^{1}H NMR (500 MHz, DMSO): δ=1.17 (br s, 6H, 10CH_3, 6CH_3^*), 1.20 (s, 3H, 11CH_3), 1.24–1.33 (m, 1H, 7CH_2^*), 1.49–1.64 (m, 5H, 8CH_2, 4CH_2^*, 7CH_2^*), 1.57, 1.64 (2×s, 6H, 10, 11CH_3^*), 1.58 (s, 3H, 6CH_3), 1.98–2.06 (m, 4H, 7CH_2, 8CH_2^*), 2.06 (s, 3H, 1CH_3), 2.10 (s, 3H, 1CH_3^*), 2.14 (q, J=7.2 Hz, 2H, 4CH_2), 2.44 (t, J=7.4 Hz, 2H, 3CH_2), 2.53 (t, J=7.4 Hz, 2H, 3CH_2^*), 2.61 (t, J=6.3 Hz, 1H, 9C*H*O), 2.63 (t, J=6.3 Hz, 1H, 5C*H*O*), 5.04–5.08 (m, 2H, 5C*H*, 9C*H**) ppm. ^{13}C NMR (125 MHz, DMSO): δ=15.6 (6*C*H_3), 16.1 (6*C*H_3^*), 17.4 (10*C*H_3^*), 18.5 (10*C*H_3), 21.8 (4*C*H_2), 22.5 (4*C*H_2^*), 24.6 (11*C*H_3), 23.4 (8*C*H_2^*), 25.4 (11*C*H_3^*), 26.8 (8*C*H_2), 29.6 (1*C*H_3, 1*C*H_3^*), 35.7 (7*C*H_2), 38.2 (7*C*H_2^*), 39.3 (3*C*H_2^*), 42.6 (3*C*H_2), 57.5 (10$(CH_3)_2$*C*O), 60.1 (6CH_3*C*O*), 61.5 (5*C*HO*), 62.7 (9*C*HO), 123.3 (5*C*H), 123.7 (9*C*H*), 130.9 (10$(CH_3)_2$*C**), 134.5 (6CH_2*C*CH_3), 207.5 (2*C*O*), 208.0 (2*C*O) ppm. GC–MS (EI) 70 eV, *m/z* (rel int. %): 210 $[M]^+$ (3), 192 $[M-H_2O]^+$ (3), 177 (2), 167 (2), 149 (5), 138 (10), 127 (10), 109 (15), 95 (30), 85 (50), 71 (30), 59 (25), 43 (100).

4.6.2. *E*-6,10-Dimethyl-9,10-epoxy-5-undecen-2-one (7). To a solution of bromohydrine **24** (196 mg, 0.673 mmol) in abs MeOH (5 ml) was added K_2CO_3 (92.9 mg, 0.673 mmol) and the mixture was stirred for 0.5 h at room temperature. The solution was diluted with water (2 ml) and extracted with CH_2Cl_2 (10 ml). The extract was washed with saturated NaCl (6 ml), dried (Na_2SO_4), and concentrated. The reaction product was purified by flash chromatography on SiO_2 with PE/EE [5:1] to give product **7** (82.0 mg, 0.390 mmol, 58%) as colorless oil. ^{1}H NMR (500 MHz, DMSO): δ=1.18, 1.20 (2×s, 6H, 10, 11CH_3), 1.50–1.55 (m, 2H, 8CH_2), 1.58 (s, 3H, 6CH_3), 1.99–2.06 (m, 2H, 7CH_2), 2.06 (s, 3H, 1CH_3), 2.10–2.16 (m, 2H, 4CH_2), 2.45 (t, J=7.3 Hz, 2H, 3CH_2), 2.62 (t, J=6.3 Hz, 1H, 9C*H*O), 5.08–5.11 (m, 1H, 5C*H*) ppm. ^{13}C NMR (125 MHz, DMSO): δ=15.7 (6*C*H_3), 18.6 (10*C*H_3), 21.9 (4*C*H_2), 24.6 (11*C*H_3), 26.9 (8*C*H_2), 29.7 (1*C*H_3), 35.8 (7*C*H_2), 42.7 (3*C*H_2), 57.5 (10$(CH_3)_2$*C*O), 62.8 (9*C*HO), 123.4 (5*C*H), 134.6 (6CH_2*C*CH_3), 208.0 (2*C*O) ppm. EIMS 70 eV, *m/z* (rel int. %): 210 $[M]^+$ (0.1), 192 $[M-H_2O]^+$ (1), 134 (6), 109 (12), 95 (19), 85 (32), 81 (19), 71 (19), 67 (14), 59 (27), 43 (100).

4.6.3. Mixture of *Z*-6,10-dimethyl-9,10-epoxy-5-undecen-2-one (10) and *Z*-6,10-dimethyl-5,6-epoxy-9-undecen-2-one (8). Nerylacetone **2** (194 mg, 1.00 mmol) and MCPBA (276 mg, 1.20 mmol) were reacted to give **10** and **8** as an inseparable (77:23) mixture (59 mg, 0.281 mmol, 28%). ^{1}H NMR (500 MHz, DMSO): δ=1.20, 1.22 (2×s, 9H, 10, 11CH_3, 6CH_3^*), 1.39–1.57 (m, 5H, 8CH_2, 7, 4CH_2^*), 1.58, 1.64 (2×s, 6H, 10, 11CH_3^*), 1.65 (s, 3H, 6CH_3), 1.69–1.75 (m, 1H, 4CH_3^*), 2.02–2.09 (m, 4H, 7CH_2, 8CH_2^*), 2.06 (s, 3H, 1CH_3), 2.10 (s, 3H, 1CH_3^*), 2.14 (q, J=7.5 Hz, 2H, 4CH_2), 2.44 (t, J=7.3 Hz, 2H, 3CH_2), 2.56 (t, J=7.5 Hz, 2H, 3CH_2^*), 2.65 (t, J=6.3 Hz, 2H, 9C*H*O, 5C*H*O*), 5.06–5.12 (m, 2H, 5C*H*, 9C*H**) ppm. ^{13}C NMR (125 MHz, DMSO): δ=17.4 (10*C*H_3^*), 18.5 (10*C*H_3), 21.7 (4*C*H_2), 21.9 (6*C*H_3^*), 22.3, 23.7 (4*C*H_2^*, 8*C*H_2^*), 23.1 (6*C*H_3), 24.7 (11*C*H_3), 25.4 (11*C*H_3^*), 26.8 (8*C*H_2), 28.0 (7*C*H_2), 29.7 (1*C*H_3, 1*C*H_3^*), 32.5 (7*C*H_2^*), 39.6 (3*C*H_2^*), 42.9 (3*C*H_2), 57.6 (10$(CH_3)_2$*C*O), 60.3 (6CH_2*C*CH_3^*), 62.7 (9*C*HO), 63.4 (5*C*HO*), 123.8 (9*C*H*), 124.3 (5*C*H), 131.1 (10*C*$(CH_3)_2^*$), 134.6 (6CH_2*C*CH_3), 207.7 (2*C*O*), 208.0 (2*C*O) ppm. GC–MS (EI) 70 eV, *m/z* (rel int. %): 210 $[M]^+$ (3), 192 $[M-H_2O]^+$ (4), 177 (3), 167 (3), 149 (5), 134 (10), 124 (15), 109 (15), 95 (30), 85 (55), 71 (25), 59 (30), 43 (100).

4.6.4. *Z*-6,10-Dimethyl-9,10-epoxy-5-undecen-2-one (10). Bromohydrine **25** (877 mg, 3.01 mmol) was used to give product **10** (511 mg, 2.43 mmol, 81%). ^{1}H NMR (500 MHz, DMSO): δ=1.20, 1.22 (2×s, 6H, 10, 11CH_3), 1.47–1.57 (m, 2H, 8CH_2), 1.65 (s, 3H, 6CH_3), 2.06 (s, 3H, 1CH_3), 2.09 (t, J=7.7 Hz, 2H, 7CH_2), 2.10–2.16 (m, 2H, 4CH_2), 2.44 (t, J=7.3 Hz, 2H, 3CH_2), 2.65 (t, J=6.3 Hz, 1H, 9C*H*O), 5.08 (t, J=7.1 Hz, 1H, 5C*H*) ppm. ^{13}C NMR (125 MHz, DMSO): δ=18.5 (10*C*H_3), 21.7 (4*C*H_2), 23.1 (6*C*H_3), 24.7 (11*C*H_3), 26.8 (8*C*H_2), 28.0 (7*C*H_2), 29.7 (1*C*H_3), 42.9 (3*C*H_2), 57.6 (10$(CH_3)_2$*C*O), 62.7 (9*C*HO), 124.3 (5*C*H), 134.6

($6CH_2CCH_3$), 208.0 (2*C*O) ppm. FTIR (ATR): 2962 (m), 2925 (m), 1715 (vs), 1377 (v), 1360 (m), 1161 (w), 1122 (w), 679 (w), 626 (w) cm^{-1}. EIMS 70 eV, *m/z* (rel int. %): 210 $[M]^+$ (4), 192 $[M-H_2O]^+$ (4), 177 (2), 167 (1), 149 (3), 138 (6), 134 (10), 124 (9), 121 (7), 109 (13), 95 (17), 85 (31), 71 (24), 59 (21), 57 (8), 43 (100). Anal. Calcd for $C_{13}H_{22}O_2$ (210.3): C, 74.24; H, 10.54. Found: C, 72.77; H, 10.64.

4.6.5. *E*-3,7-Dimethyl-6,7-epoxy-2-octenol (13). To the well-stirred ice bath cooled (1–3 °C) suspension of geraniol **3** (1.70 ml, 9.70 mmol) and $NaHCO_3$ solution in water (0.25 M, 280 ml, 5.88 g) was added dropwise MPPA solution in water (100 ml, 2.73 g, 0.15 M). The reaction was stirred at 1–3 °C for 2 h. The mixture is then saturated (NaCl) and extracted with ethyl ether (4×80 ml). The organic layer was washed with NaCl solution (2×40 ml), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on SiO_2 with *n*-He/DE [3:2, then 1:1, 1:2] to give **13** (0.34 g, 2.0 mmol, 72%) as major product, **14** (96 mg, 0.564 mmol, 20%), and nonreacted geraniol **3** (1.07 g, 6.94 mmol, 29% conversion). ^{1}H NMR (500 MHz, DMSO): δ=1.19, 1.22 (2×s, 6H, 7, 8CH_3), 1.53–1.58 (m, 2H, 5CH_2), 1.59 (s, 3H, 3CH_3), 2.02–2.10 (m, 2H, 4CH_2), 2.65 (t, *J*=6.3 Hz, 1H, 6C*H*O), 3.94 (t, *J*=5.8 Hz, 2H, 1CH_2O), 4.46 (t, *J*=5.2 Hz, 1H, O*H*), 5.28–5.30 (m, 1H, 2C*H*) ppm. ^{13}C NMR (125 MHz, DMSO): δ=16.0 (3*C*H_3), 18.6, 24.6 (7, 8*C*H_3), 26.8 (5*C*H_2), 35.6 (4*C*H_2), 57.5 (1*C*H_2), 57.6 (7*C*$(CH_3)_2$), 62.7 (6*C*HO), 125.7 (2*C*H), 134.9 ($3CH_3$*C*CH_2) ppm. EIMS 70 eV, *m/z* (rel int. %): 170 (0.1) $[M]^+$, 152 (0.2) $[M-H_2O]^+$, 137 (1), 109 (7), 97 (10), 85 (34), 81 (38), 71 (28), 67 (19), 59 (60), 57 (29), 55 (21), 43 (64), 41 (100).

4.6.6. *E*-3,7-Dimethyl-2,3-epoxy-6-octenol (14). To the suspension of geraniol **3** (1.70 ml, 9.70 mmol) and NaOH solution in water (0.25 M, 280 ml) was added dropwise MPPA (4.55 g, 25.0 mmol) in 100 ml water. The reaction was stirred at room temperature for 5 h. This suspension was saturated with NaCl solution and extracted with ether (3×80 ml). The organic layer was washed with NaCl solution (2×40 ml), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on SiO_2 with *n*-He/DE [1:1] to give **14** as a colorless oil (680 mg, 3.99 mmol, 97%) and nonreacted geraniol **3** (861 g, 5.58 mmol, 43% conversion). ^{1}H NMR (500 MHz, DMSO): δ=1.19 (s, 3H, 3CH_3), 1.34–1.40 (m, 1H, 4CH_2), 1.52–1.58 (m, 1H, 4CH_2), 1.57, 1.64 (2×s, 6H, 7, 8CH_3), 1.99–2.03 (m, 2H, 5CH_2), 2.77 (t, *J*=5.6 Hz, 1H, 2C*H*O), 3.41–3.45 (m, 1H, 1CH_2), 3.50–3.54 (m, 1H, 1CH_2), 4.85 (t, *J*=5.4 Hz, 1H, O*H*), 5.09 (t, *J*=6.6 Hz, 1H, 6C*H*) ppm. ^{13}C NMR (125 MHz, DMSO): δ=16.4 (3*C*H_3), 17.4 (7*C*H_3), 23.3 (5*C*H_2), 25.4 (8*C*H_3), 38.1 (4*C*H_2), 59.7 (3O*C*CH_3), 59.8 (1*C*H_2), 62.6 (2*C*HO), 123.7 (6*C*H), 131.0 (7*C*$(CH_3)_2$) ppm. EIMS 70 eV, *m/z* (rel int. %): 152 (1) $[M^+-H_2O]^+$, 139 (1), 121 (2), 109 (22), 95 (11), 82 (19), 69 (38), 67 (38), 55 (24), 43 (68), 41 (100).

4.6.7. *Z*-3,7-Dimethyl-6,7-epoxy-2-octenol (15). To the well-stirred ice bath cooled (1–3 °C) suspension of nerol **4** (771 mg, 5.00 mmol) and $NaHCO_3$ solution in water (2.94 g, 35 mmol, 140 ml) was added dropwise MPPA solution in water (50 ml, 1.37 g, 7.52 mmol). The reaction was stirred at 1–3 °C for 2 h. The mixture is then saturated (NaCl) and extracted with ethyl ether (4×40 ml). The organic layer was washed with NaCl solution (2×20 ml), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on SiO_2 with PE/Et_2O [1:2] to give **15** (221 mg, 1.30 mmol, 53%) as major product, **16** (105 mg, 0.617 mmol, 25%), and nonreacted nerol **4** (392 mg, 2.54 mmol, 49% conversion). ^{1}H NMR (500 MHz, DMSO): δ=1.19, 1.23 (2×s, 6H, 7, 8CH_3), 1.41–1.59 (m, 2H, 5CH_2), 1.70 (s, 3H, 3CH_3), 2.10 (t, *J*=7.7 Hz, 2H, 4CH_2), 2.65 (t, *J*=6.4 Hz, 1H, 6C*H*O), 3.89–3.95 (br m, 2H, 1CH_2O), 4.46 (t, *J*=5.1 Hz, 1H, O*H*), 5.31 (t, *J*=6.3 Hz, 1H, 2C*H*) ppm. ^{13}C NMR (125 MHz, DMSO): δ=18.5 (7*C*H_3), 23.1 (3*C*H_3), 24.6 (8*C*H_3), 27.0 (5*C*H_2), 28.2 (4*C*H_2), 57.2 (1*C*H_2), 57.6 (7*C*$(CH_3)_2$), 62.7 (6O*C*HCH_2), 126.7 (2*C*H), 135.2 (3O*C*CH_3) ppm. EIMS 70 eV, *m/z* (rel int. %): 152 $[M-H_2O]^+$ (0.1), 137 (1), 109 (7), 97 (8), 85 (31), 81 (24), 71 (24), 67 (19), 59 (56), 43 (61), 41 (100).

4.6.8. *Z*-3,7-Dimethyl-2,3-epoxy-6-octenol (16). To the suspension of nerol **4** (0.77 g, 4.99 mmol) and NaOH solution in water (1.40 g, 140 ml) was added dropwise MPPA (2.28 g, 12.5 mmol) in 50 ml water. The reaction was stirred at room temperature for 5 h. This suspension was saturated with NaCl solution and extracted with ether (3×40 ml). The organic layer was washed with NaCl solution (2×20 ml), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on SiO_2 with DE/PE [1:1] to give **16** as a colorless oil (656 mg, 3.85 mmol, 96%), and nonreacted nerol **4** (154 mg, 0.998 mmol, 80% conversion). ^{1}H NMR (500 MHz, DMSO): δ=1.23 (s, 3H, 3CH_3), 1.36–1.42 (m, 1H, 4CH_2), 1.48–1.53 (m, 1H, 4CH_2), 1.57, 1.65 (2×s, 6H, 7, 8CH_3), 1.99–2.07 (m, 2H, 5CH_2), 2.77 (dd, *J*=4.9, 5.9 Hz, 1H, 2C*H*O), 3.39–3.43 (m, 1H, 1CH_2), 3.53–3.57 (m, 1H, 1CH_2), 4.87 (t, *J*=5.6 Hz, 1H, O*H*), 5.07–5.11 (m, 1H, 6C*H*) ppm. ^{13}C NMR (125 MHz, DMSO): δ=17.3 (7*C*H_3), 21.9 (3*C*H_3), 23.7 (5*C*H_2), 25.4 (8*C*H_3), 32.8 (4*C*H_2), 59.5 (1*C*H_2), 59.9 (3O*C*CH_3), 63.8 (2*C*HO), 123.7 (6*C*H), 131.0 (7*C*$(CH_3)_2$) ppm. EIMS 70 eV, *m/z* (rel int. %): 152 $[M-H_2O]^+$ (1), 137 (1), 109 (23), 95 (10), 82 (19), 69 (38), 67 (36), 55 (22), 43 (66), 41 (100).

4.6.9. Geranylacetone-5,6,9,10-diepoxide (22). To a solution of geranylacetone **1** (580 g, 2.98 mmol) in CH_2Cl_2 (15 ml) was added slowly MCPBA (1.34 g, 5.96 mmol) and stirred at room temperature for 4 h. Then the mixture was filtered, washed with saturated $NaHCO_3$ solution (2×10 ml), and the water layer was extracted with CH_2Cl_2 (2×10 ml). The combined organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on SiO_2 with EE/PE [1:2] to give **22** (276 mg, 1.22 mmol, 41%) as a colorless oil and as a (1:1) mixture of diastereomers. ^{1}H NMR (500 MHz, DMSO): δ=1.19, 1.20, 1.22 (3×s, 9H, 6, 10, 11CH_3), 1.40–1.47 (m, 1H, 7CH_2), 1.48–1.58 (m, 2H, 8CH_2), 1.69–1.70 (m, 3H, 4, 7CH_2), 2.10 (s, 3H, 1CH_3), 2.57 (dt, *J*=7.3, 4.2 Hz, 2H, 3CH_2), 2.63–2.66 (m, 1H, 9C*H*O), 2.67 (t, *J*=6.3 Hz, 1H; 5C*H*O) ppm. ^{13}C NMR (125 MHz, DMSO): δ=16.1, 16.2, 18.4 (10, 11*C*H_3), 22.4 (4*C*H_2), 24.2, 24.3 (8*C*H_2), 24.5 (6*C*H_3), 29.6 (1*C*H_3), 34.8, 35.0 (7*C*H_2), 39.4 (3*C*H_2), 57.4, 57.6 (10$(CH_3)_2$*C*O), 60.0 (6CH_2*C*CH_3), 61.3, 61.5 (5*C*HO), 62.6 (9*C*HO), 207.5, 207.6 (2*C*O) ppm. t_R=14.20 min; GC–MS (EI) 70 eV, *m/z* (rel int. %): 226

$[M]^+$ (5), 211 (10), 143 (15), 126 (25), 109 (20), 95 (20), 84 (80), 71 (30), 55 (10), 43 (100). t_R=14.24 min; GC–MS (EI) 70 eV, *m/z* (rel int. %): 226 $[M]^+$ (5), 211 (10), 143 (15), 127 (15), 109 (20), 95 (15), 84 (95), 71 (30), 59 (10), 43 (100). In addition 40% of an inseparable (60:40) mixture of **7** and **21** was isolated (see above for details of characterization).

4.6.10. Nerylacetone-5,6,9,10-diepoxide (23). Nerylacetone **2** (450 mg, 2.31 mmol) and MCPBA (1.04 g, 4.62 mmol) were reacted to give **23** (204 mg, 0.901 mmol, 39%). ^{1}H NMR (500 MHz, DMSO): δ=1.20, 1.21, 1.22 (3×s, 9H, 6, 10, 11CH_3), 1.48–1.58 (m, 5H, 4, 7, 8CH_2), 1.60–1.76 (m, 1H, 4CH_2), 2.10 (s, 3H, 1CH_3), 2.57 (t, *J*=7.3 Hz, 2H, 3CH_2), 2.66–2.71 (m, 2H, 5, 9C*H*O) ppm. ^{13}C NMR (125 MHz, DMSO): δ=18.3, 18.4, 21.8, 21.9 (10, 11*C*H$_3$), 22.2 (4*C*H$_2$), 24.5 (6*C*H$_3$), 24.6, 29.0 (7, 8*C*H$_2$), 29.6 (1*C*H$_3$), 39.5 (3*C*H$_2$), 57.6, 57.6 (10(CH$_3$)$_2$*C*O), 60.1, 60.2 (6CH$_2$*C*CH$_3$), 62.6, 62.7, 62.7, 62.8 (5, 9*C*HO), 207.5, 207.6 (2*C*O) ppm. GC–MS (EI) 70 eV, *m/z* (rel int. %): 226 $[M]^+$ (2), 211 (2), 127 (70), 109 (15), 95 (15), 84 (100), 71 (30), 59 (10), 43 (95). In addition 7% of an inseparable (77:23) mixture of **10** and **8** was isolated (see above for details of characterization).

4.6.11. *E*-9-Bromo-6,10-dimethyl-10-hydroxy-5-undecen-2-one (24). A mixture of *N*-bromosuccinimide (NBS) (452 mg, 2.60 mmol) in THF/H$_2$O (68:32, 19 ml) was added dropwise over a 40 min period to the ice bath cooled (0 °C) solution of geranylacetone **1** (428 mg, 2.20 mmol) in THF/H$_2$O (68:32, 21 ml). The stirring was continued at 0 °C for 10 min. The mixture was then diluted with $CHCl_3$ (26 ml) and the organic layer was washed with saturated NaCl (20 ml), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on SiO_2 with PE/EE [3:1] to give product **24** (207 mg, 0.711 mmol, 32%) as yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ=1.34 (2×s, 6H, 10, 11CH_3), 1.62 (s, 3H, 6CH_3), 1.77–1.80 (m, 1H, 8CH_2), 1.97–2.00 (m, 1H, 8CH_2), 2.08–2.16 (m, 2H, 7CH_2), 2.15 (s, 3H, 1CH_3), 2.27–2.32 (m, 3H, 4CH_2, 10O*H*), 2.48 (t, *J*=7.3 Hz, 2H, 3CH_2), 3.94 (dd, *J*=1.8, 11.4 Hz, 1H, 9C*H*Br), 5.13–5.18 (m, 1H, 5C*H*) ppm. ^{13}C NMR (125 MHz, DMSO): δ=15.9 (6*C*H$_3$), 22.4 (4*C*H$_2$), 26.0 (10*C*H$_3$), 26.5 (11*C*H$_3$), 30.0 (1*C*H$_3$), 31.9 (8*C*H$_2$), 38.1 (7*C*H$_2$), 43.6 (3*C*H$_2$), 70.5 (9*C*HBr), 72.5 (10(CH$_3$)$_2$*C*OH), 124.3 (5*C*H), 134.5 (6CH$_2$*C*CH$_3$), 208.7 (2*C*O) ppm.

4.6.12. *Z*-9-Bromo-6,10-dimethyl-10-hydroxy-5-undecen-2-one (25). Nerylacetone **2** of 1.30 g (6.69 mmol) was used to give product **25** (1 g, 3.43 mmol, 51%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=1.34, 1.36 (2×s, 6H, 10, 11CH_3), 1.67 (s, 3H, 6CH_3), 1.74–1.82 (m, 1H, 8CH_2), 1.97–2.04 (m, 1H, 8CH_2), 2.15 (br s, 4H, 1CH_3, 10O*H*), 2.27–2.35 (m, 4H, 4, 7CH_2), 2.46–2.49 (m, 2H, 3CH_2), 3.64 (dd, *J*=1.7, 11.4 Hz, 1H, 9C*H*Br), 5.15 (t, *J*=7.2 Hz, 1H, 5C*H*) ppm. ^{13}C NMR (125 MHz, DMSO): δ=22.4 (4*C*H$_2$), 23.2, 26.0, 26.5 (6, 10, 11*C*H$_3$), 30.0 (1*C*H$_3$), 30.5 (8*C*H$_2$), 32.1 (7*C*H$_2$), 43.9 (3*C*H$_2$), 70.7 (9*C*HBr), 72.5 (10(CH$_3$)$_2$*C*OH), 125.2 (5*C*H), 134.6 (6CH$_2$*C*CH$_3$), 208.7 (2*C*O) ppm. FTIR (ATR): 3431 (s), 2968 (m), 2930 (m), 1708 (vs), 1363 (s), 1160 (m), 962 (w), 631 (s) cm^{-1}. MS (EI), *m/z* (%): 290/292 (0.1) [M$^+$], 272 (1), 193 (30), 135 (66), 93 (24), 69 (38), 43 (100), 18 (9). Anal. Calcd for $C_{13}H_{23}BrO_2$ (291.2): C, 53.61; H, 7.96; Br, 27.44. Found: C, 53.56; H, 8.14; Br, 27.79.

4.6.13. *E*-3,7-Dimethyl-2,3,6,7-bisepoxy-2-octenol (27). To a well-stirred and cooled (1–3 °C) mixture of geraniol **3** (309 mg, 2.00 mmol) in sodium bicarbonate solution (0.5 M, 8 ml) was added in small portions MCPBA (1.34 g, 7.8 mmol) and stirred at room temperature for 1 h. Then a mixture was saturated with NaCl, extracted with CH_2Cl_2 (10 ml), dried, and concentrated. The residue was purified by flash chromatography on SiO_2 with EE/PE [3:1] to give **27** (229 mg, 1.23 mmol, 61%) as a colorless oil and as a (1:1) mixture of diastereomers. ^{1}H NMR (500 MHz, DMSO): δ=1.20, 122 (2×s, 9H, 3, 7, 8, CH_3), 1.45–1.55 (m, 3H, 5CH_2, 4CH_2), 1.62–1.69 (m, 1H, 4CH_2), 2.68 (t, *J*=6.0 Hz, 1H; 6C*H*), 2.80 (q, *J*=5.6 Hz, 1H, 2C*H*O), 3.41–3.47 (m, 1H, 1CH_2O), 3.51–3.54 (m, 1H, 1CH_2O), 4.84–4.87 (m, 1H, O*H*) ppm. ^{13}C NMR (125 MHz, DMSO): δ=16.3, 18.4, 24.5 (7, 8, 3CH_3), 24.1, 24.2 (5CH_2), 34.7, 34.8 (4CH_2), 57.5, 57.7 (7*C*(CH$_3$)$_2$), 59.4 (3O*C*CH$_3$), 59.7 (1CH_2), 62.4, 62.5, 62.6 (2, 6*C*HO) ppm. t_R=13.45 min; GC–MS (EI) 70 eV, *m/z* (rel int. %): 186 $[M]^+$ (1), 155 (3), 125 (15), 111 (30), 93 (10), 84 (85), 71 (60), 59 (35), 43 (100), 29 (10). t_R=13.56 min; GC–MS (EI) 70 eV, *m/z* (rel int. %): 186 $[M]^+$ (1), 155 (2), 125 (10), 111 (25), 97 (10), 84 (100), 71 (55), 59 (40), 43 (90), 29 (10).

4.6.14. *Z*-3,7-Dimethyl-2,3,6,7-bisepoxy-2-octenol (28). To a cooled (0 °C) mixture of nerol **4** (0.35 ml, 2.0 mmol) in CH_2Cl_2 (4 ml) was added under nitrogen dropwise over a 1 h period MCPBA (1.35 g, 7.8 mmol) in CH_2Cl_2 (14 ml) and stirred at room temperature overnight. After then, a second portion of MCPBA (897 mg, 5.2 mmol) in CH_2Cl_2 (8 ml) was added and the mixture was stirred for 3 h. Then a mixture was filtered, washed with saturated $NaHCO_3$ solution (20 ml), and the water layer was extracted with CH_2Cl_2 (20 ml). The combined organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on SiO_2 with EE/PE [3:1] to give **28** (188 mg, 1.01 mmol, 51%) as a colorless oil and as (1:1) mixture of diastereomers. ^{1}H NMR (500 MHz, DMSO): δ=1.20, 1.22, 1.23, 1.24 (4×s, 9H, 3, 7, 8, CH_3), 1.46–1.65 (m, 4H, 4CH$_2$, 5CH_2), 2.69 (t, *J*=4.4 Hz, 1H, 6C*H*), 2.78–2.81 (m, 1H, 2C*H*O), 3.42–3.48 (m, 1H, 1CH_2O), 3.52–3.58 (m, 1H, 1CH_2O), 4.89–4.89 (m, 1H, O*H*) ppm. ^{13}C NMR (125 MHz, DMSO): δ=18.3, 18.4, 21.8, 24.5 (3, 7, 8CH_3), 24.6, 24.7 (5CH_2), 29.3 (4CH_2), 57.6 (7*C*(CH$_3$)$_2$), 59.4 (1CH_2), 59.7, 59.8 (3*C*OCH$_3$), 62.5, 62.6, 63.7, 63.8 (2, 6*C*HO) ppm. R_f=13.45 min; 186 $[M]^+$ (10), 155 (3), 126 (10), 111 (30), 108 (30), 84 (95), 71 (65), 59 (40), 43 (100), 29 (10). t_R=13.55 min; GC–MS (EI) 70 eV, *m/z* (rel int. %): 186 $[M]^+$ (5), 155 (2), 125 (5), 111 (25), 108 (15), 84 (100), 71 (55), 59 (40), 43 (85), 29 (10).

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