Chapter 9

Summary

In the present work several full-length cDNAs and cDNA fragments of sesquiterpene synthases from the plant *Solidago canadensis* were isolated. The cDNAs were isolated by screening a λ-phage cDNA library with a probe generated by degenerate primer PCR. Another strategy to isolated full-length cDNAs proceeded by RACE-PCR. Four of the isolated full-length cDNAs were functionally expressed in *E. coli*. The products catalytically formed by the heterologously expressed sesquiterpene synthases were generated by incubation with the general substrate farnesyl diphosphate followed by a product identification by GC-MS and GC with enantioselective stationary phase. The functionally expressed sesquiterpene synthases were identified as the following:

- (+)-germacrene D-synthase ((+)GDS) producing exclusively the (+)-germacrene D enantiomer (90%) and some unidentified minor side products.

- (–)-germacrene D-synthase ((–)GDS) producing exclusively the (–)-germacrene D enantiomer (90%) and also some unidentified minor side products.

- α-gurjunene-synthase (αGS) producing α-gurjunene (81.3%), next to γ-gurjunene (8.8%) and bicyclogermacrene (7.1%).

- cascarilladiene-synthase producing cascarilladiene (77.3%) and δ-selinene (14.5%) as side product.

The two germacrene D synthases represent the main sesquiterpenes in the essential oil of *Solidago canadensis*. α-Gurjunene is an essential sesquiterpene intermediate en route to cyclocolorenone, which is another major component of the essential oil of *Solidago canadensis*. Cascarilladiene is a minor sesquiterpene component, which was not known before to be existent in *Solidago canadensis*. The pH optima and the enzyme kinetic data were determined for each of the sesquiterpene synthases. The pH optima of the enzymes were around pH 7.5 and the K_M were between 6 and 16 μM, which are values generally found for sesquiterpene synthases.

This is the first time, that two sesquiterpene synthases, each producing one enantiomer of the same sesquiterpene, in this case germacrene D, were isolated from one plant species. Furthermore the full-length sequences of (+)GDS and (–)GDS share very high homology with amino acid sequence identity of 85%. This opened the possibility to get more insight into the enantiospecific catalytic action of the two enzymes. Comparative homology modeling was performed on the known structure of *epi*-aristolochene synthase, followed by substrate docking experiments by docking the germacrenyl cation intermediate common to the both synthases. In this way five amino acid residues were determined, which presumably are responsible in the enantiospecific catalysis of both germacrene D-synthases.

By isolating the two enantiospecific (+)GDS and (–)GDS in addition to successful heterologous expression in *E. coli*, the necessary amount of enzyme can be produced for X-ray crystallography experiments leading to the 3-D structure of the two synthases. This would give a more detailed insight into the enantiospecifically working active sites.

Furthermore a group of four sesquiterpene synthase sequences from *Solidago canadensis* with sequence identity above 84% were obtained, of which three were iso-

lated within this work ((+)GDS, (–)GDS and αGS) next to the known germacrene A-synthase [Prosser et al., 2002] showing that only 10% to 15% changes in the amino acid sequence are sufficient for product diversity of sesquiterpene synthases.