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Chiral chemodiversity and its role for biological activity. Some observations from studies on insect/insect and insect/plant relationships

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Abstract: Many compounds occur in Nature in both enantiomeric forms. This *chiral chemodiversity* is common among certain classes of natural products whereas other classes of compounds usually occur in only one of the enantiomeric forms. Thus triterpenoids and steroids belong to the same enantiomeric family. On the other hand, many of the lower terpenoids (mono- sesqui-, and diterpenoids) are present in both of their enantiomeric forms. The chiral chemodiversity in relation to the biological functions of the compounds will be discussed with reference to recent results on the enantiomeric composition of monoterpenes from various natural sources. Focus will be on the role of the enantiomeric composition of α-pinene in the host plant, *Picea abies* (L.) Karst., of the spruce bark beetle, *Ips typographus*. Research on the biological functions of chiral compounds requires test samples of very high enantiomeric purity. Synthetic and analytical methods are important and our current studies on such methods are referred to.

INTRODUCTION

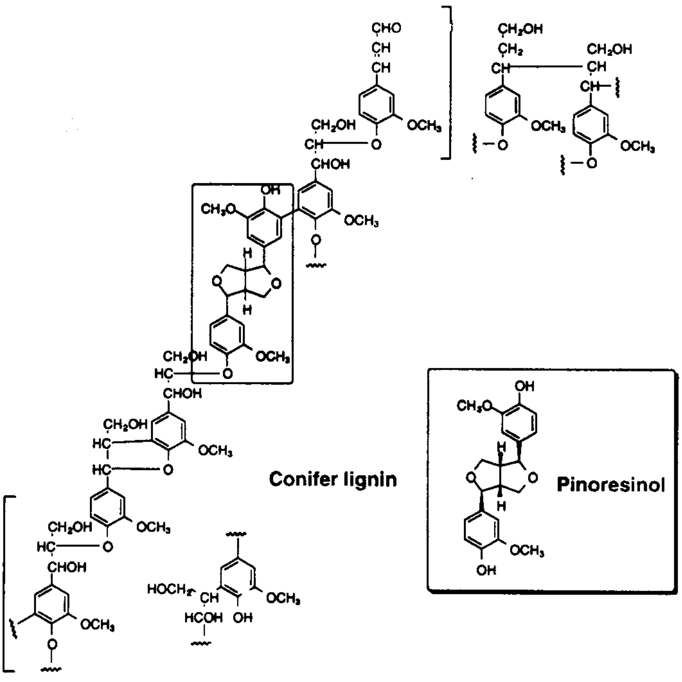
The title of this account is rather broad. However, it is intended to provide some information on our recent research on insect/insect and insect/plant relationships and some general ideas and conclusions on chiral chemodiversity.

The chemodiversity in Nature is most fascinating. Up to now more than 100,000 low molecular weight natural products have been described (1) and many more are to come. An obvious question is: why does Nature exhibit such a chemodiversity? Most probably because the compounds are essential for the life processes of the producing organisms and specificity has been a driving force in the evolution. It is also possible that compounds, which to our present knowledge have no obvious function, were once essential in the earlier evolution of the organism but now remain as "metabolic fossils".

Most of the asymmetric natural products occur in an optically active form. The background for the establishment of the natural absolute configurations of the self-replicating RNA/DNA or the peptide/protein systems is still not known, although these molecules are responsible for the chirality in Nature (2). The origin of this chirality is a topic for different hypotheses, none of which have been properly supported by convincing experiments or scientific observations. One important factor, which constitutes the foundation for the control of enantiomeric and diastereomeric purity, is inherent in the biosynthesis of nucleic acids and proteins. A sort of proof-reading is intrinsic in the biosyntheses of the compounds.

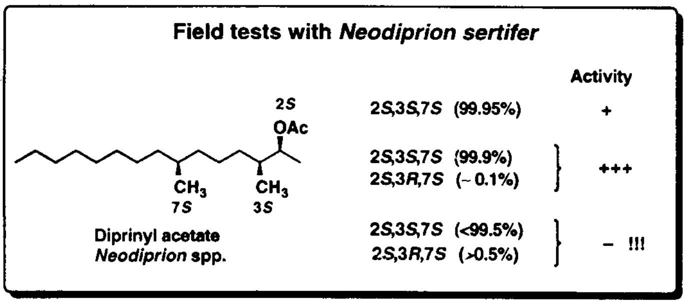
Enantiomerically and diastereomerically pure compounds are significant for essential signals of the fundamental life processes (e.g. various hormones). Many natural products, which are not of this type, are also formed enantiomerically pure. However, very often natural products occur as mixtures of enantiomers. Racemic asymmetric natural products are much less common.

The plant lignins are interesting examples of racemic asymmetric natural products. It is of particular interest to note that such a racemic lignin polymer co-occurs with the optically active monomeric structure elements of lignan type such as the lignan pinoresinol of pine trees (Scheme 1).



Scheme 1

The importance of chirality for bioactivity is well known. The pheromone field offers excellent examples where chirality plays an important role in the expression of bioactivity. One particular case from our own research work is the very active sex pheromones of saw flies – *Neodiprion* and *Diprion* species. The pheromone constituents of the pine sawflies have been studied by several groups – particularly by Matsumura and Coppel in the USA and by our group in Sweden (for references see 3). We have found that there is a very delicate tuning of the diastereomeric composition of the pheromone components in order to obtain biological activities (Scheme 2).



Field tests with *Neodiprion sertifer*

	Activity
2*S*,3*S*,7*S* (99.95%)	+
2*S*,3*S*,7*S* (99.9%) 2*S*,3*R*,7*S* (~0.1%)	+++
2*S*,3*S*,7*S* (<99.5%) 2*S*,3*R*,7*S* (>0.5%)	– !!!

Scheme 2

This particular case demonstrates the importance of having access to enantiomerically and diastereomerically pure compounds for biological studies.

CHIRAL CHEMODIVERSITY AMONG TERPENOIDS

Chiral chemodiversity in Nature is particularly displayed among the terpenoids, which represent a large family of natural products. More than 23,000 different terpenes have been described in the literature (4). The enzyme involved in the key step of the biosynthesis of the higher terpenoids from sesquiterpenes to triterpenes and steroids, has recently been structurally determined (5). This fact opens up possibilities both for mechanistic studies and for controlling the biosynthesis of these types of compounds, many of which are essential for life processes.

Most of the terpenoids are optically active and they may occur in Nature in either of the two enantiomeric forms. They can also occur as mixtures of enantiomers. This situation is known for mono- and sesquiterpenes. On the other hand, the triterpenes and steroids occur in Nature in only one of the enantiomeric forms.

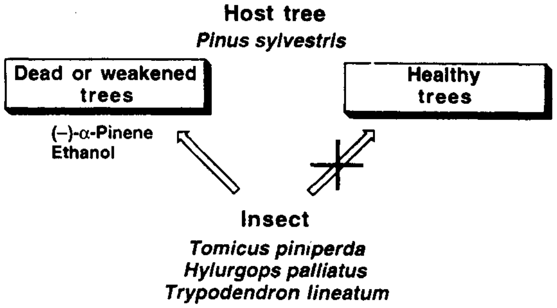
The co-occurrence of the two enantiomeric forms of monoterpenes has been realized already at the beginning of this century. The biosynthetic and ecological implications of this chiral chemodiversity are now open for scientific explorations, since proper tools for determination of enantiomeric compositions have been developed.

There are different possibilities for the biosynthesis of compounds, which occur as mixtures of enantiomers:

- non-enantiospecific transformation (same enzyme is operating for the formation of each enantiomer)
- the compound is formed as a racemate but is a substrate for further enzymatic transformations and one enantiomer is more readily transformed
- two different enzymes are operating.

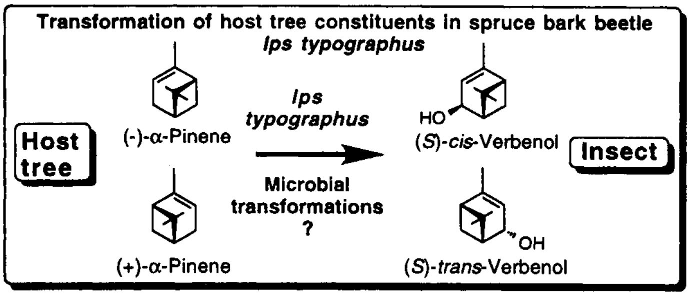
ENANTIOMERIC COMPOSITIONS OF MONOTERPENES AND INSECT / HOST INTERACTIONS

We became interested in the enantiomeric compositions of terpenes from various forest trees because of their role in the interaction between some pest insects and their host trees (6). Some forest pest insects have a preference to attack dead or weakened trees. In the cases shown in Scheme 3, the host substances, mainly α-pinene together with ethanol, produced by the weakened tree, were found to be the signal for the primary aggregation (6, 7).



Scheme 3

An important example of insect/host interaction is that of the spruce bark beetle, *Ips typographus*, and its host tree, the common spruce, *Picea abies* (L.) Karst. The female bark beetle is primarily attracted by a host tree and the beetle transforms the host terpene (–)-α-pinene to *cis*-verbenol (Scheme 4), which is an active constituent of the aggregation pheromone (8, 9). This aggregation pheromone attracts further individuals, both males and females, to the suitable breeding host. The transformation of the monoterpene is probably performed by microorganisms in the intestinal tract of the insect. It has been shown by Renwick *et al.* (8) and later by Byers (10), that when the insects are exposed to (–)-α-pinene, *cis*-verbenol is formed, whereas when it is exposed to (+)-α-pinene, *trans*-verbenol is formed. *trans*-Verbenol is not a component of the aggregation pheromone and, together with verbenone, it seems to inhibit aggregation in field experiments.



Scheme 4

This observation suggests that trees in which the phloem lacks (–)-α-pinene, may be resistant to beetle attack. In order to test this hypothesis we started an investigation of the enantiomeric compositions of monoterpenes in spruce trees. For this purpose we have been engaged in the development of GC-methods. A Polish research team – Sybilska and Jurczac in Warsaw – had shown that GC-columns coated with α-cyclodextrin in formamide separated the enantiomers of α-pinene and β-pinene (11). We investigated this method and found that water is necessary for the separation. Micro-packed silica columns, impregnated with a solution of α-cyclodextrin in formamide and water, were very efficient for the separation of several of the common monoterpene hydrocarbons (12). Since the carrier gas has to be saturated with water and for other practical reasons, this method is not very suitable for capillary applications but can be used for preparative purposes.

During the period of these studies very efficient GC-methods for enantiomeric separations of monoterpenes were developed by the groups of Schurig and König (13). They used alkylated cyclodextrins as a stationary phase. Capillary GC-columns based on these findings are now commercially available and have been used in our studies.

In order to be able to perform an analysis of a complex mixture in a short time, we developed a 2D-GC analytical technique (14). The individual components are first separated in a conventional GC-capillary column and these compounds are then transferred to a second column for the enantiomeric separation. In order to avoid overlap, our system has two parallel chiral columns and the various monoterpenes are transferred by a microswitch system onto either of these columns depending on their retention properties. The system is computer controlled and uses pneumatic microvalves. We have used this system for the investigation of enantiomeric compositions of natural products from various sources. More recently, the solid phase micro-extraction technique (15) has improved the sensitivity of our GC analysis. This technique is highly recommended for head-space analyses in ecological studies.

Our results from the investigation of the enantiomeric compositions of common spruce, *Picea abies* (14, 16), and Scots pine, *Pinus sylvestris* (17), were most surprising. It is previously known that the composition of individual components of the tree varies in different parts of the tree, e.g. the amount of pinene in wood, bark, root, and needles. It was also well known that there are large individual variations partly due to genetic

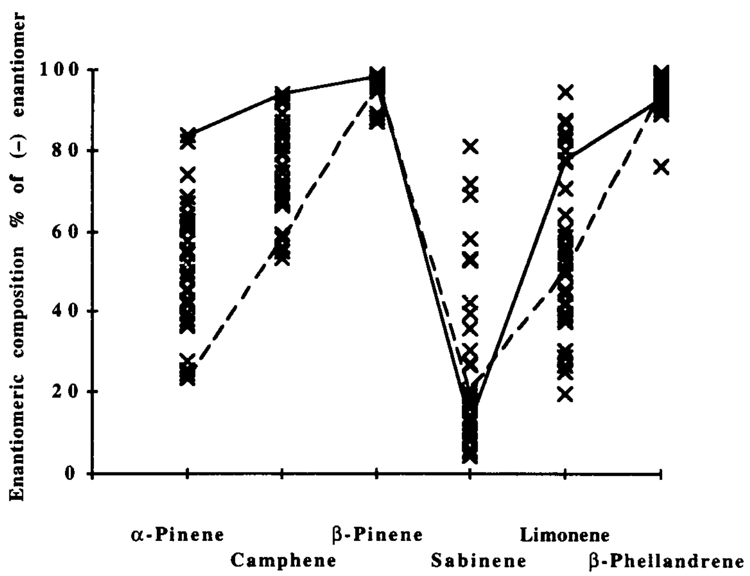


Figure 1. Enantiomeric compositions of the main chiral monoterpenes in the xylem of 36 individual trees, *Picea abies* (L.) Karst. The lines are connecting the values of two individual trees, one (straight line) representing a tree with the highest and the other (dotted line) a tree with the lowest relative ratio of (–)-α-pinene.

reasons. But it is now also clear that there are large variations in the enantiomeric compositions of different components both regarding different individual trees and different parts of a tree. Thus, we have found a variation of the enantiomeric ratio of α-pinene from 90 % of the (–)-form to almost 80 % of the (+)-form.

The large individual variations in enantiomeric compositions of the main chiral monoterpene hydrocarbons in the xylem of *Picea abies* are shown in Figure 1.

We are now collaborating with forest geneticists to investigate genetic relationships between trees based on enantiomeric compositions. One important task is to identify the genes responsible for the production of the various terpenes. For this approach we need information about the biosynthetic pathways leading to the various monoterpenes and in particular to α-pinene.

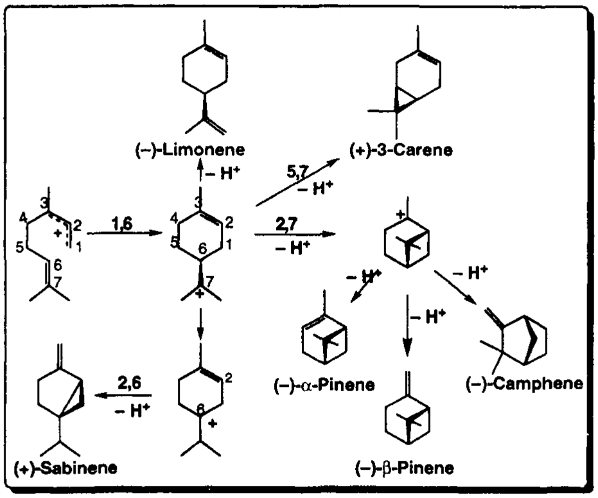
MONOTERPENE BIOSYNTHESIS

The previous view on the biosynthesis of monoterpenes is that the cyclization of the geranyl pyrophosphate proceeds *via* a common cationic precursor, which in turn is further transformed into the various monoterpenes (Scheme 5). Labelling experiments have supported such a hypothesis.

In recent studies, mainly by Croteau and his group in Oregon, USA, the enzymes involved in the biosyntheses of limonene, pinene and carene have been described (18 – 21). These studies demonstrate that specific cyclases are operating, one specific cyclase for each compound (Scheme 6). This is also supported by our studies on enantiomeric compositions.

Our conclusions are based on the fact that one would expect a similar enantiomeric ratio for biosynthetically related compounds if they are formed from the same intermediate. However, this is not the case. One significant observation from our studies is that there are drastic differences in enantiomeric compositions of the α- and β-pinene from the same source (Figure 1). This clearly indicates that there are different enzymes involved in the syntheses of these two compounds. Similarly, the enantiomeric ratios of limonene, camphene, 3-carene, and α-pinene from one individual are very different despite the fact that they are considered to be formed *via* a similar chiral cationic intermediate.

Further support for the hypothesis that the two enantiomers of α-pinene and other monoterpenes are produced by different enzymes comes from the fact that the enantiomeric composition varies in different tissues and in different individual trees. The only correlation between the enantiomeric compositions of the different monoterpenes is that there are individual trees with high enantiomeric ratios of monoterpenes of the (–)-family and others with low values for such ratios.



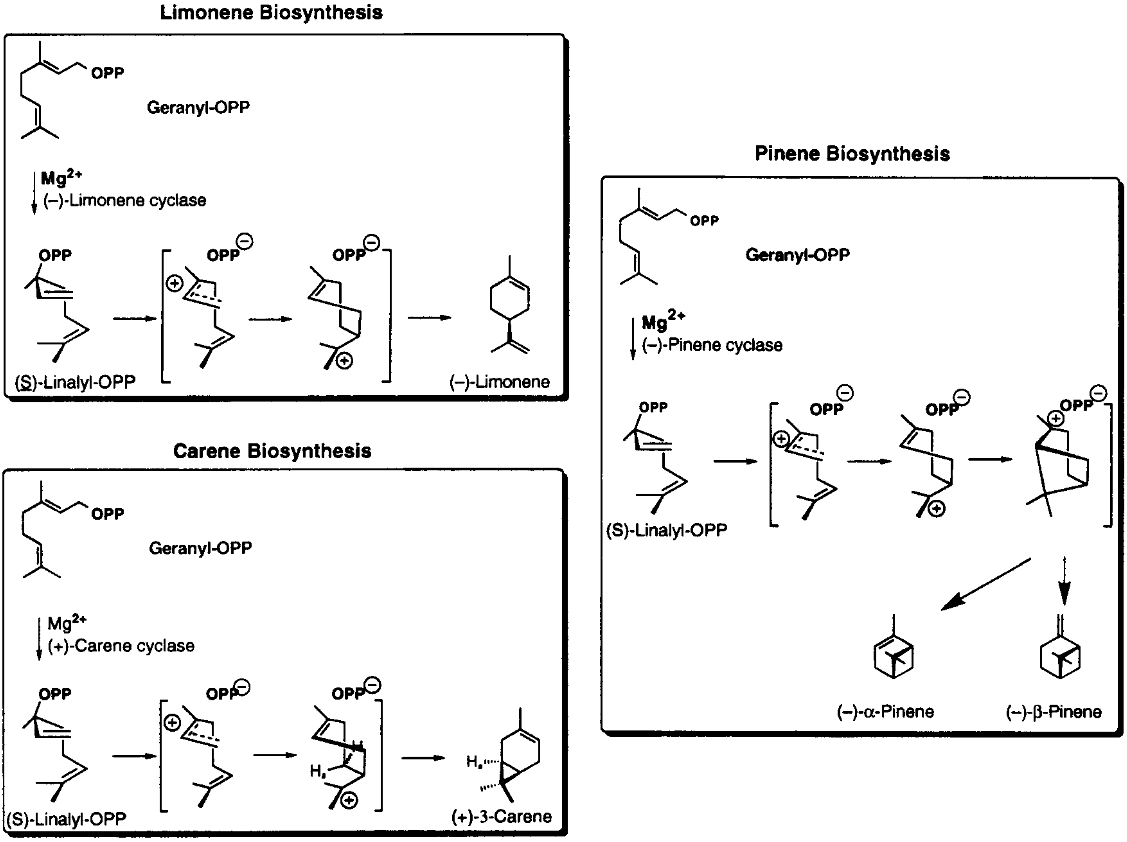
Scheme 5

It is interesting to note that the different monoterpene cyclases, which produce monoterpenes of the same enantiomeric family may be structurally related. They have to fold the linalyl pyrophosphate in a similar way and generate a carbocationic intermediate of a similar shape as indicated in Scheme 6. Further studies on the structures and functions of these monoterpene cyclases will reveal interesting details of carbocationic mechanisms and rearrangements.

The conclusions from the enzyme studies by Croteau and from our own studies on enantiomeric compositions are that there are different enzymes for the production of the two enantiomers. This indicates that it should be possible, by genetic techniques, to modify the biosynthetic routes in order to alter the monoterpene compositions in plants. Thus, we may be able to reach our goal to obtain a common spruce, which does not produce (–)-α-pinene and, therefore, is not predetermined for bark beetle attacks.

CONCLUSIONS

From our studies we have obtained information about the enantiomeric compositions as well as the biosyntheses of monoterpenes in forest trees. Current studies with the aim to induce spruce bark beetle resistance by manipulating the biosynthesis of α-pinene in the host tree have been presented. Our studies on insect pheromones and insect/plant interactions have also initiated research on the use of enzymes in organic synthesis, when there has been a need for components with very high enantiomeric purities. We hope to apply this knowledge to other enzyme systems and in particular to terpene cyclases, which may provide powerful tools for organic synthesis. Such knowledge will also be important for genetic studies as well as for plant breeding, in order to improve pest resistance or to increase the production of a desired terpene.



Scheme 6

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