Enantiomeric Composition of Monoterpene Hydrocarbons in Different Tissues of Norway Spruce, *Picea abies* (L.) Karst. A Multi-dimensional Gas Chromatography Study

Anna-Karin Borg-Karlson,[a] Mikael Lindström,[a,b] Torbjörn Norin,[a] Monika Persson[a] and Irena Valterová[a,c]

[a] Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden, [b] (Present address) Firmenich SA, Research Laboratories, P.O. Box 239, CH-1211 Geneva 8, Switzerland and [c] Visiting research fellow from Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague, Czechoslovakia

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The enantiomeric composition of seven chiral monoterpene hydrocarbons in *Picea abies* (L.) Karst. has been determined using a multi-dimensional GC-system with one DB-WAX and two permethylated β-cyclodextrin capillary columns. Different tissues, e.g. xylem of trunk and branch, phloem, oleoresin of branch, and needles were analyzed.

The relative amounts and enantiomeric ratios of the monoterpene hydrocarbons differed considerably between different tissues. However, (+)- and (−)-α-pinene and (−)-β-pinene were the main compounds in all tissues except in the needles, where (−)-camphene predominated, followed by (−)-α-pinene and (−)-limonene. On the average, the constituents of the phloem and oleoresin had a higher enantiomeric purity than those of the xylem and needles.

Turpentine from thermo-mechanical pulping (TMP) of Norway spruce wood was also analyzed and the composition of the monoterpene hydrocarbon fraction as well as the enantiomeric ratios were found to be similar to the average of the values obtained from the xylem samples.

The importance of the chiral composition of the monoterpenes as a base for biological and biosynthetic considerations is discussed.

Dedicated to Professor Salo Gronowitz on the occasion of his 65th birthday.

The individual variations in the monoterpene hydrocarbon content in oleoresin of Scots pine, *Pinus sylvestris* L. and in needles of Norway spruce *Picea abies* (L.) Karst. has been analyzed and compared quantitatively and qualitatively within and between populations and clones.[1–5] The variation in relative amounts of monoterpene hydrocarbon constituents has been found to be large between, but not within, clones. The enantiomeric ratios of α-pinene in the oleoresins of individual spruce trees and the variation of this ratio between clones have already been reported.[6,7]

In this study the analyses of the enantiomeric compositions of the six major chiral monoterpene hydrocarbons in xylem, phloem, needles and oleoresin of two individuals of Norway spruce are presented.

Turpentine from thermo-mechanical pulping (TMP) of Norway spruce wood, mixed with minor quantities of hardwoods, was also analyzed. In this process, the wood chips are treated with steam and the composition of the TMP-turpentine constituting the organic phase of the steam distillate depends on the average content of monoterpene hydrocarbons in spruce trees from the area where the trees were harvested. The hardwoods (birch and aspen) do not contribute to the monoterpene constituents in the turpentine.[8]

Since the introduction of enantioselective gas chromatography columns based on cyclodextrins[9] or their derivatives,[10,11] analyses of the enantiomeric composition of compounds in complex mixtures without prior derivatization have been made possible. The enantioselective columns commercially available are very efficient for enantiomeric separation but not very selective for diastereomers or structural isomers. Thus the complex mixtures of compounds in biological samples require pre-fractionation to prevent overlapping. However, pre-fractionation in combination with chiral separation can be performed in a multi-dimensional gas chromatography (MD-GC) system. Commercially available MD-GC systems are all based on the use of an arrangement for cutting and regulating gas flows called 'live-T.' Since we required a versatile system, where megabore columns or packed columns could be used together with one or two

capillary columns, a system based on micro-valves was constructed. It is described below.

Materials and methods

Spruce samples. The samples were collected from two trees, 25 and 40 years old, respectively, on March 14–15, 1991, just before the spring season (outdoor temperature, ca. 5°C), at Österskär, 35 km northeast of Stockholm, Sweden. Fresh 5-year-old needles (20–40 needles) from the main stem branch developed in 1986 were cut at millimetre intervals and the phloem was detached under a microscope. A xylem plug (6 × 40 mm) from the trunk was drilled at 1.5 m height. The plug and the xylem from the branch (15–20 mm) were cut in 1 mm slices. Resin samples were collected in the spring from the main stem branch (from the resin ducts in the cortical tissue) using 0.5 µl microcaps. All samples were extracted fresh in 0.5 ml of hexane (pa Merck) for 24 h and filtered through 150–200 mg of silica gel, Matrex silica 90–130 µm, pore size 30 Å (trade name AMICON), prior to GC analyses.

Reference chemicals and TMP-turpentine. The samples of (+)-α-pinene and (−)-α-pinene, (−)-β-pinene, (+)-sabinene, (+)-3-carene, (+)- and (−)-limonene, and (+)- and (−)-camphene were all commercially available. Racemic β-phellandrene and β-pinene were gifts from Firmenich SA. (+)-β-Phellandrene was prepared from (−)-limonene.[12] Tricyclene was identified by GC–MS, and (−)-3-carene was isolated from *Nasutitermes ephratae* termites.[13]

The TMP-turpentine sample was a gift from the TMP plant of Svenska Cellulosa AB (SCA) in Ortviken near Sundsvall (500 km north of Stockholm). The TMP-turpentine sample was diluted with hexane and filtered through silica gel prior to the GC analyses.

The MD-GC system. An MD-GC system was used in this study. Two Varian 3400 gas chromatographs were connected with a heated interface and two Valco micro-valves (1/32″) were installed according to the drawing in Fig. 1. The GC system was equipped with split/splitless injectors and flame ionizing detectors. Deactivated fused silica capillaries were used as retention gaps and for the connection of the different valves with each other and with the detectors. A DB-WAX (Carbowax-type) column was used for the pre-separation in the first gas chromatograph (Fig. 2, A). The heart-cut times were set from a normal chromatogram, i.e. the sample was injected in GC A (3,

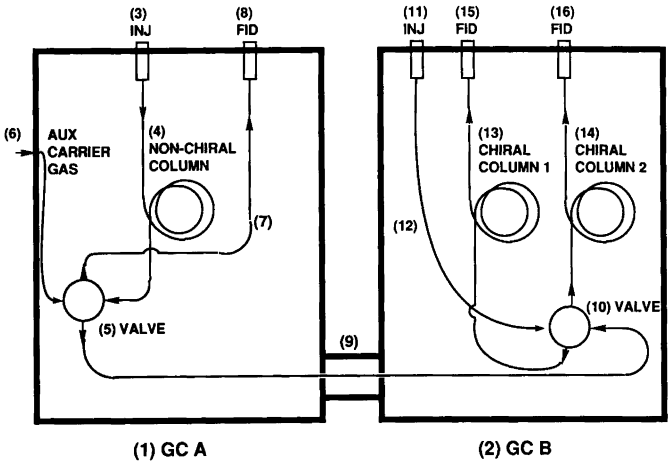

Fig. 1. A schematic drawing of the multi-dimensional gas chromatography (MD-GC) system used in this study. For a description see the text: (1) GC A. Temperature programme 40°C for 1 min, followed by 11 min at 65°C (rate between 40 and 65°C: 50°C min^{-1}) and about 20 min at 130°C (rate between 65 and 130°C: 20°C min^{-1}). (2) GC B. 55°C isothermal. (3) Varian split/splitless injector 1075; injector temperature 150°C; splitless mode; opened after 6 s. (4) J&W fused silica DB-WAX column; 30 m, i.d. 0.25 mm, coating layer 0.25 µm, carrier-gas flow 0.82 ml min^{-1}. (5) Valco 1/32″ valves Valcon E rotor with removable polyimide liner and polyimide ferrules (1/32″ FSR adapter) in combination with 1/32″ nuts were used for connections of the columns in the valves; temperature limits are ambient up to 225°C. (6) Auxiliary helium carrier gas; the connection between the metal carrier gas transline and valve (5) is made of 1.0 m deactivated fused silica tubing i.d. 0.25 mm. (7) Fused silica tubing, 1.0 m, i.d. 0.1 mm connected between the valve (5) and the detector (8); to fit into the valve a short piece of fused silica tubing i.d. 0.25 mm, was glued to the tubing with i.d. 0.1 mm. The narrow tubing has a resistance which corresponds to the resistance in a 30 m, i.d. 0.25 mm tubing. This arrangement made the flow approximately the same in the DB-WAX column, independently of the position of the valve. The time delay for compounds passing between the valve and the detector was insignificant, which made it possible to set a cutting time for the valve from the retention times achieved from a normal GC run in GC A. (8) FID-detector, temperature 200°C. (9) Transfer line (70°C) controlled from GCA, deactivated fused silica tubing (1.0 m, i.d. 0.25 mm) was used as a connection between the valves (5) and (10). (10) Valco valve 1/32″, see also (5). (11) Varian split/splitless injector 1077, in split mode. (12) Deactivated fused silica tubing (0.5 m, i.d. 0.25 mm). (13), (14) J&W permethylated (Cyclodex B) β-cyclodextrin fused silica column (30 m, i.d. 0.25 mm, 0.25 µm coating layer), carrier gas flow 1.24–1.3 ml min^{-1}. (15), (16) FID detector, temperature 200°C.

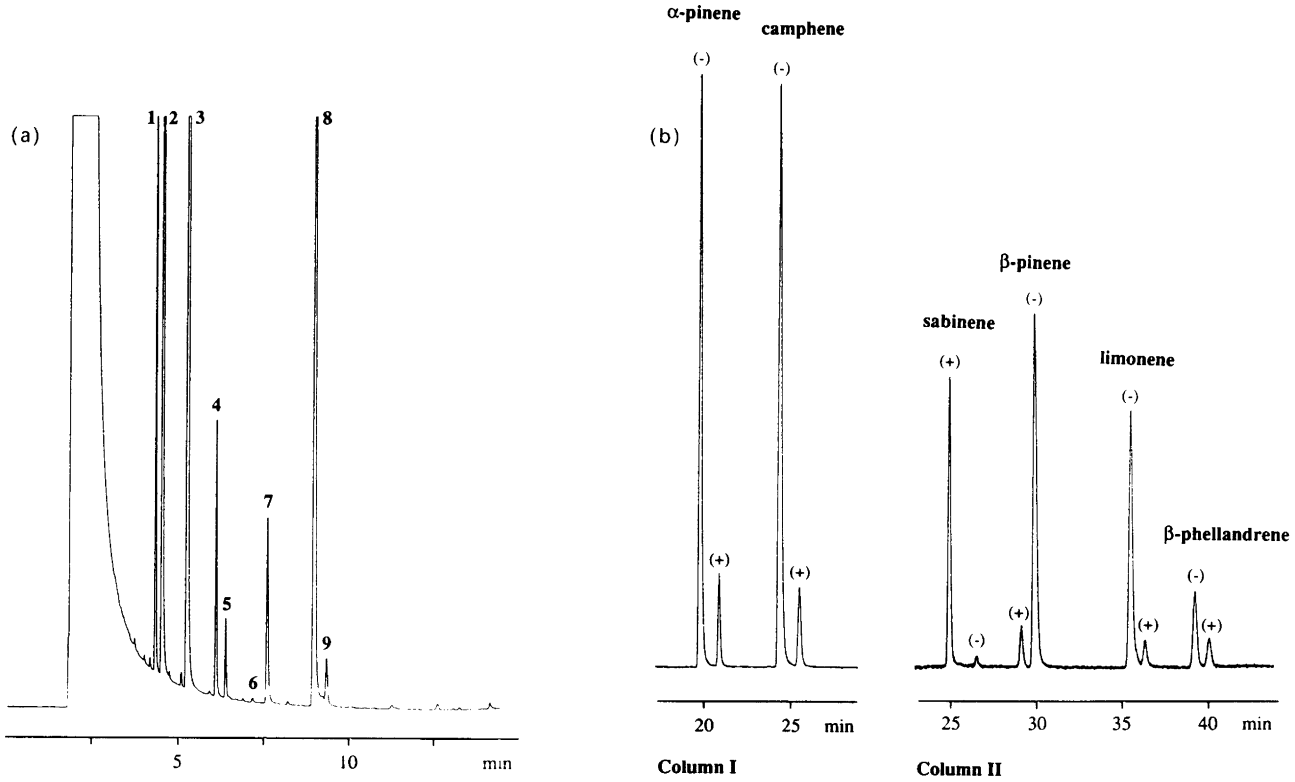

Fig. 2. Gas chromatograms from an MD-GC separation of a hexane extract of needles of Norway spruce: (a) normal GC performance of the total monoterpene fraction on a DB-WAX capillary silica column and (b) the different chiral monoterpenes separated on two enantioselective β-CD columns. For chromatographic details see the text. 1, tricyclene; 2, α-pinene; 3, camphene; 4, β-pinene; 5, sabinene; 6, 3-carene; 7, myrcene; 8, limonene; 9, β-phellandrene.

Fig. 1) and the valve (5, Fig. 1) was in a position so as to transfer all the peaks to the detector (8, Fig. 1). For the chiral analysis the valve (5, Fig. 1) was programmed according to the retention times in the first run. Short cut times (1/100 min) were set for large peaks to avoid overloading on the chiral column. To optimize the signal-to-noise ratio in the chiral column longer cut times (10/100 min) were set for small peaks. No extra sample focusing between GC A and GC B was needed since the peaks were narrow enough to give good resolution in the enantiomeric separation. In order to prevent overlapping of sabinene and camphene, two enantioselective J&W β-cyclodextrin (β-CD) columns were used in parallel for the enantiomeric separation in a second gas chromatograph (Fig. 2, B). This arrangement made it possible to transfer six monoterpenes to the chiral columns during one GC performance and still obtain good resolution. Helium was used for the activation of the valves and as a carrier and make up gas in the system. The concentration needed to determine the enantiomeric composition of a compound in a sample was about 1/1000–1/10.000 v/v. Each GC run took 40 min. The chromatograms were recorded on three Spectraphysics SP 4400 Chromjet integrators. The experimental conditions for the analyses are given in the legend to Fig. 1.

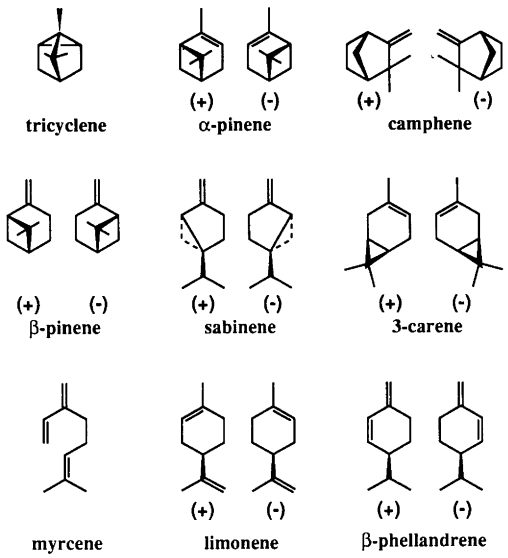

Fig. 3. The molecular structures of tricyclene, myrcene and the enantiomeric pairs of the seven chiral monoterpene hydrocarbons studied.

The elution order of the enantiomeric pairs of the six monoterpenes analyzed on the β-CD column was: α-pinene, (−/+); sabinene, (+/−); camphene, (−/+); β-pinene, (+/−); limonene, (−/+); and β-phellandrene, (−/+); see Fig. 2, A and B and for the structures Fig. 3.

The GC-retention times of the samples were compared with those of authentic compounds of natural or synthetic

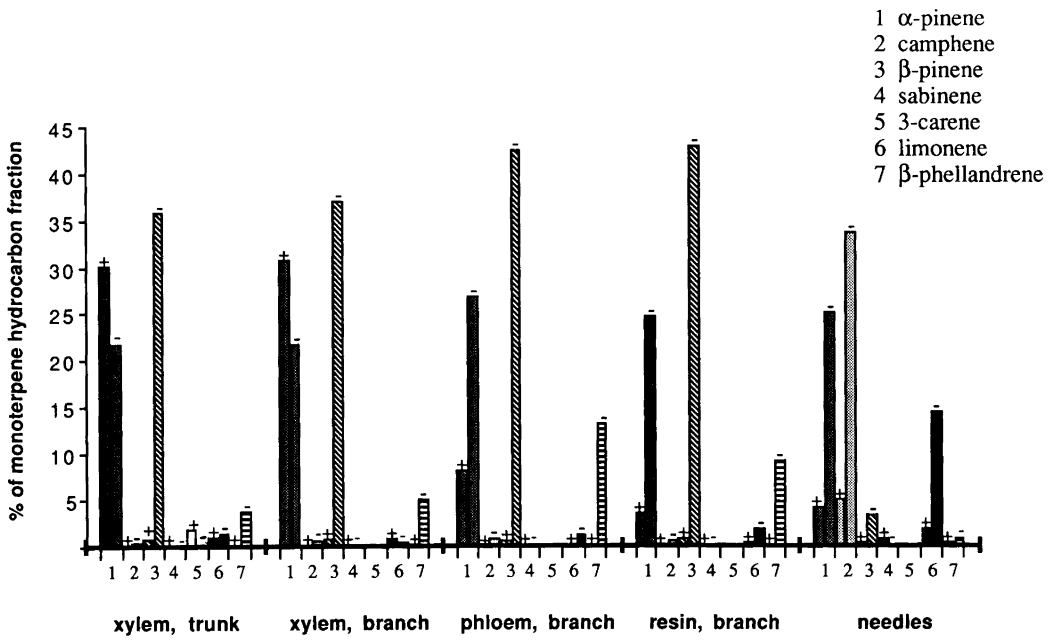

Fig. 4. Relative amounts of monoterpene hydrocarbons divided into (+)- and (−)-enantiomers in different tissues of *P. abies*. The bars represent the means of five samples from each tissue from tree No. 1. For numerical values see Table 1.

origin. The enantiomeric composition of 3-carene in the spruce samples could not be determined because insufficient amounts were present in these samples.[1,*] In the turpentine sample the enantiomeric composition of 3-carene was determined by the following procedure. The compound was isolated by preparative gas chromatography using a P-Unicam GCV, equipped with an effluent splitter (1:100), followed by injection into a micro-packed column (0.8 mm i.d. × 1.8 m) packed with Chromosorb W-AW and coated with α-cyclodextrin in a water–formamide GC system equipped with a device for saturating the carrier gas with water.[14]

Results

The results are presented in Tables 1–3, and Figs 2, 4–6.

Analyses of living spruce trees. Fig. 4 shows the relative amounts of enantiomers of monoterpene hydrocarbons, as percentages of the monoterpene hydrocarbon fractions according to GC, in different tissues in tree No. 1, whereas Table 1 lists the data for both trees.

In the xylem of the trunk, (+)-α-pinene (30% and 36% of the total amount), (−)-α-pinene (22% and 28%) and (−)-β-pinene (36% and 22%, respectively) were the main constituents, followed by small proportions of (−)-β-phellandrene, (−)-limonene, (+)-3-carene, and tricyclene.

In the needles, (−)-camphene was the main monoterpene (34% and 30% of the total amounts), followed by (−)-α-pinene (25% and 22%), (−)-limonene (14% and 19%, respectively) and tricyclene (6.3% and 5.6%). (−)-β-Pinene and (−)-β-phellandrene were present in small amounts, less than 5%.

The enantiomeric ratios are shown in Table 2 and Figs. 5 and 6. α-Pinene was found to be almost racemic in the xylem. The enantiomeric ratios {(−)-α-pinene/(+)-α-pinene} were 42/58 and 43/57 in the trunk and 41/59 and 45/55 in the four-year-old xylem of the branch. In the phloem the enantiomeric ratios of α-pinene were 77/23 and 78/22 and in the needles 86/14 and 85/15. β-Pinene was almost enantiomerically pure in all tissues: ca. 98/2 except in the needles where the ratios were 90/10 and 91/9 {(−)-β-pinene/(+)-β-pinene}. The enantiomeric ratio of β-phellandrene exceeded 99/1 in all tissues except in the needles, where the ratios were 71/29 in tree 1 and 48/52 in tree 2. The content of sabinene was very low (less than 1% of the monoterpene fraction) in some tissues and the (+)-enantiomer pre-dominated both in the trunk xylem (32/68 and 9/91) and in the needles (4/96 and 2/98). Considerable variations in the enantiomeric ratio of camphene and limonene were found both between different tissues and between the two trees.

Analyses of TMP-turpentine. The composition of the monoterpene hydrocarbon fraction of the TMP-turpentine is presented in Table 3 and Fig. 6. The major constituents were α- and β-pinene, followed by limonene and 3-carene. Camphene and β-phellandrene were present only in small amounts. β-Pinene, β-phellandrene and 3-carene were of high enantiomeric purity, whereas camphene and α-pinene were almost racemic. The

* The enantiomeric composition of 3-carene in the xylem samples from the trunks has been analyzed on a new capillary Lipodex E (dipentylbutyryl-γ-cyclodextrin, Macherey-Nagel) column (30 m, i.d. = 0.25 mm, 30°C in second gas chromatograph). The amounts of 3-carene in the samples from branches and needles were too small to be analyzed.

Table 1. Relative amounts of monoterpene hydrocarbons in different tissues of *Picea abies* (L.) Karst. The values represent relative amounts as percentages of the total monoterpene hydrocarbons according to GC. The six main enantiomeric pairs of monoterpenes are divided into (+) and (−) enantiomers plus 3-carene, tricyclene and myrcene; — = not present in detectable amounts. * = only (+)-3-carene was detected.

Compound Relative amounts	Xylem, trunk 1	Xylem, trunk 2	Xylem, branch 1	Xylem, branch 2	Phloem, branch 1	Phloem, branch 2	Oleo-resin 1	Oleo-resin 2	Needles 1	Needles 2
Tricyclene	0.30	0.40	0.30	0.70	0.20	0.30	0.20	0.30	6.30	5.60
(+)-α-Pinene	30.10	36.40	30.80	37.80	8.11	8.19	3.42	3.36	4.17	4.11
(−)-α-Pinene	21.80	28.00	21.70	31.00	26.80	28.20	24.70	30.20	25.10	22.40
(+)-Camphene	0.21	0.32	0.20	0.32	0.19	0.05	0.16	0.01	5.01	4.47
(−)-Camphene	0.39	1.13	0.44	2.94	0.65	1.33	0.54	0.99	33.70	29.90
(+)-β-Pinene	0.73	0.72	0.68	0.55	0.58	0.55	0.65	0.47	0.39	0.17
(−)-β-Pinene	35.90	21.70	37.00	16.80	42.60	24.60	42.90	26.90	3.33	1.73
(+)-Sabinene	0.18	0.32	0.13	0.11	—	0.15	0.03	0.20	0.79	1.49
(−)-Sabinene	0.08	0.03	0.05	0.02	—	—	0.67	—	0.03	0.03
3-Carene	1.84*	0.35*	—	0.25	0.12	2.20	0.10	2.40	0.08	0.12
Myrcene	0.62	1.05	1.02	0.78	1.80	2.10	3.70	4.00	2.80	5.36
(+)-Limonene	1.00	0.94	0.76	0.77	0.16	0.12	0.30	0.20	1.73	1.61
(−)-Limonene	1.29	2.36	0.31	2.81	1.24	6.78	1.70	9.70	14.40	18.5
(+)-β-Phellandrene	Trace	0.15	0.02	Trace	0.04	—	0.09	—	0.30	0.35
(−)-β-Phellandrene	3.78	4.95	4.98	3.16	13.10	19.20	17.40	19.20	0.74	0.31
Other compounds	1.78	1.18	1.62	1.99	4.41	6.23	3.44	2.17	1.14	3.85

Table 2. The amounts of (−)-enantiomers as percentages of the total amount of both (+)- and (−)-enantiomers (according to GC) of six monoterpenes in different tissues of two (1 and 2) *Picea abies* (L.) Karst. trees. The numbers in parentheses are calculated standard deviations from five samples.

Compound (−)-Enantiomer	Xylem, trunk 1	Xylem, trunk 2	Xylem, branch 1	Xylem, branch 2	Phloem, branch 1	Phloem, branch 2	Oleo-resin 1	Oleo-resin 2	Needles 1	Needles 2
α-Pinene	42.0 (1.92)	43.4	41.4 (2.58)	45.0 (3.16)	76.8 (7.5)	77.5 (9.2)	87.8	90	85.8 (0.52)	84.5 (0.6)
Camphene	65.8 (1.74)	77.8	69.2 (2.92)	90.3 (1.16)	77.7 (3.14)	96.2 (5.6)	77.3	99	87.0 (0.46)	87.0 (0.58)
β-Pinene	98.0 (0.43)	96.8	98.2 (0.071)	96.8 (0.11)	98.6 (0.15)	97.8 (0.55)	98.5	98.3	89.6 (2.09)	90.8 (5.75)
Sabinene	31.6 (29.1)	8.5	26.1 (4.47)	12.9 (10.9)	—	—	67.1	—	3.62 (2.64)	1.98 (1.36)
Limonene	56.1 (21.36)	71.4	29.5 (5.30)	78.6 (2.73)	88.2 (2.72)	98.2 (1.60)	85.1	98.0	89.3 (1.23)	92.0 (3.97)
β-Phellandrene	>99.9 (0.1)	97.5	99.6 (0.89)	>99.9	99.7 (0.63)	<99.9	99	>99.9	71.4 (10.3)	47.6 (2.05)

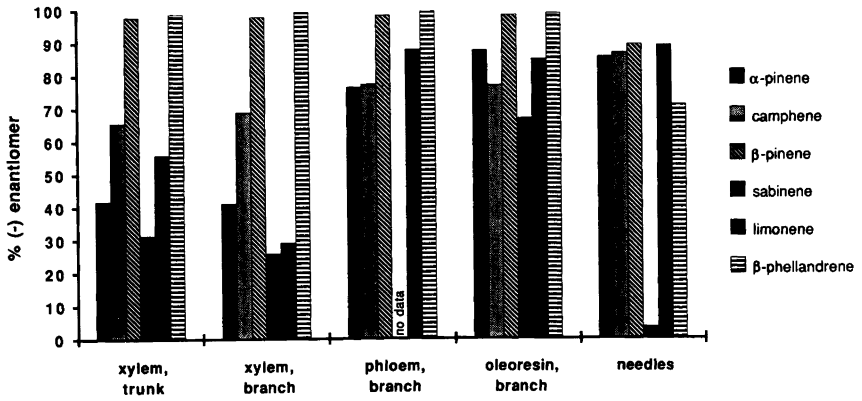

Fig. 5. Enantiomeric composition of the six main chiral monoterpene hydrocarbons in different tissues of *P. abies*. The bars represent the means of five samples from each tissue from tree No. 1. The composition is given as percentage of the (−)-enantiomer of the monoterpene hydrocarbon. For numerical values see Table 2.

(−)-enantiomers were pre-dominant in β-pinene, limonene and β-phellandrene. 3-Carene was present as the pure (+)-enantiomer.

Discussion

The relative abundance of the individual enantiomers of the major monoterpene hydrocarbons is shown in Figs. 4 and 5. The differences in enantiomeric composition of α-pinene, camphene, β-pinene, β-phellandrene, sabinene and limonene in the tissues xylem, phloem, needles and resin may be the results of alternative biosynthetic pathways, possibly operating to different extents in the xylem, in the cortical tissues and in the needles. A knowledge of configurational relationships of the monoterpene constituents is expected to be a useful base for biogenetic considerations. However, further analyses are needed before any hypothetical biosynthetic pathways can be discussed. The seven major chiral monoterpene hydrocarbons (Fig. 4) must be considered as fourteen separate compounds in a hypothetical biosynthetic scheme. A more extensive study concerning trees of different genetic origin is in progress.

Most of the monoterpene hydrocarbons are present in higher enantiomeric purity in the needles than in the xylem (Fig. 5). There is a high degree of similarity regarding enantiomeric ratios between the xylem of the living trees investigated and the turpentine (Tables 1–3 and Fig. 6). The TMP-turpentine is of interest as a valuable source of chemicals such as (−)-β-pinene, (−)-β-phellandrene and (+)-3-carene, some of which can be used as important auxiliaries or building blocks in asymmetric organic syntheses (the 'chiron' approach)[15] or as starting materials for the production of flavours and fragrances.

Many species of forest pest insects are known to have their own preferences for attack sites; e.g. different parts of a tree are attractive to different insect species.[16] The Norway spruce sawfly *Pristiphora abietina* (Htg.) attacks

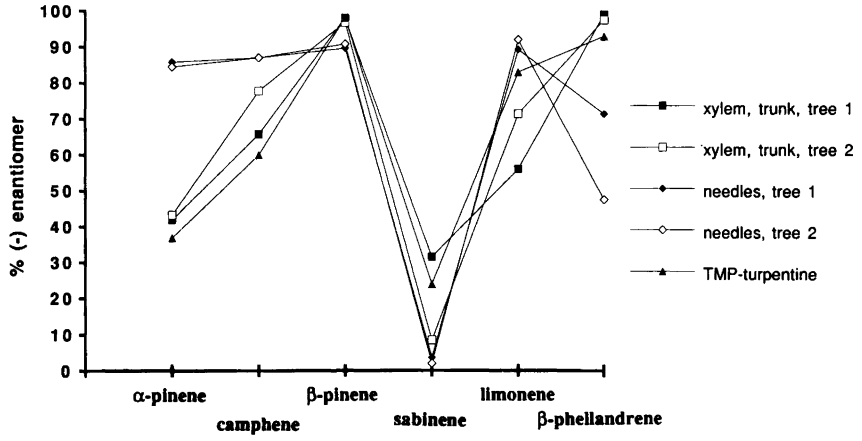

Fig. 6. Enantiomeric composition of the six main chiral monoterpene hydrocarbons in trunk xylem and needles of the two *P. abies* trees, compared with corresponding data for TMP-turpentine. The compositions are expressed as percentages of the (−)-enantiomer of each monoterpene. For numerical values see Tables 2 and 3.

Table 3. Relative amounts and enantiomeric ratios of the main monoterpene hydrocarbons in swedish TMP-turpentine from *Picea abies* (L.) Karst. The monoterpenes are listed in elution order on DB-WAX.

Compound	Proportion as % of total amount according to GC	Enantiomeric ratio(+)/(−)
α-Pinene	54.3	63/37
Camphene	1.1	40/60
β-Pinene	25.3	2/98
Sabinene	0.5	76/24
3-Carene	7.1	>99.5/<0.5
Myrcene	1.3	—
Limonene	7.9	17/83
β-Phellandrene	2.5	7/93

the youngest shoots, the sprout ends, and the larvae feed inside the needles; *Pachynematus scutellatus* (Htg.) also feeds on new shoots.[16] These young shoots contain almost no monoterpenes.[5,17] The false spruce wedworm *Cephalcia abietis* (L.), on the other hand, does not attack the youngest shoots, but the fully developed older ones, which contain large amounts of monoterpene hydrocarbons. These insects feed on the needles and the larvae move from younger twigs to older twigs at the top of the tree.[16] A number of beetle species such as the spruce longicorn, *Tetropium ssp. castaneum* (L.) and the pine sawer, *Monochamus sutor* (L.) feed on the xylem of the trunk,[16] while the six-toothed spruce bark beetle *Pityogenes chalcographus* (L.) prefers thinner parts of the branches.

Apart from the morphological differences of different tissues in the tree, the enantiomeric ratio may have an effect on the attraction that the tree exerts on various feeding and breeding insects. Two enantiomers of a chiral compound may act physiologically as two different compounds. The differences in enantiomeric composition of monoterpenes in the various tree tissues may thus influence the insect in its choice of host tree or site of attack. The absolute quantity of specific components in the resin produced by the tree may also be important for the efficiency of an attack and the extent of the injury.[18]

One of the economically most harmful insects in *P. abies*, the spruce bark beetle, *Ips typographus* (L.) feeds on the phloem of the trunk, especially on cut logs and on ageing or environmentally stressed trees. The insects need (−)-α-pinene as a precursor for the production of their aggregation pheromone component (*S*)-*cis*-verbenol.[6] The absence (−)-α-pinene in a spruce tree, may decrease or inhibit the biosynthesis of aggregation pheromones in bark beetles feeding on that tree.

A synergistic effect is shown in the attraction of the pine weevil, *Hylobius abietis* (L.) to the roots of pine trees, when α-pinene is combined with ethanol. This effect seems to be partly inhibited by limonene.[18] In spite of these observations, pine trees that have a high limonene content[4] do not seem to exert a repellent effect on the beetles.[18,19] This is probably due to variations in the relative amount or the enantiomeric ratio of limonene both within and between individual trees.

Our investigation shows that the relative amounts and enantiomeric ratios of monoterpene hydrocarbons differ between different tissues and individuals (Figs. 4 and 5). Thus, there seems to be a chemical basis for the selective preferences of forest insect species for different tissues in the tree. However, our analyses reflect only the proportions, whereas the absolute amounts of those compounds involved in insect attraction may also be of great importance.

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References

1. Yazdani, R., Rudin, D., Alden, T., Lindgren, D., Harbom, B. and Ljung, K. *Hereditas 7* (1982) 261.
2. Yazdani, R. and Nilsson, J. E. *Scand. J. For. Res. 1* (1986) 85.
3. Baradat, Ph. and Yazdani, R. *Scand. J. For. Res. 3* (1988) 25.
4. Yazdani, R. In: Lindgren, D., Ed., *Prevenances and Forest Tree Breeding for High Latitudes–Proceedings of the Frans Kempe Symposium*, Umeå 1986.
5. Bufler, U., Seusert, G. and Jüttner, F. *Environmental Pollution 68* (1990) 367.
6. Lindström, M., Norin, T., Birgersson, G. and Schlyter, F. *J. Chem. Ecol. 15* (1989) 541.
7. Lindström, M. Ph. D. Thesis, KTH (1989).
8. Kimland, B. and Norin, T. *Svensk Papperstidn. 75* (1972) 311.
9. Koscielski, T., Sybilska, D. and Jurczak, J. *J. Chromatogr. 280* (1983) 131.
10. Juvanscz, Z., Alexander, G. and Szeltli, J. *J. High Res. Chromatogr. Chromatogr. Commun. 10* (1987) 105.
11. Schurig, V. and Nowotny, H.-P. *Angew. Chem. 29* (1990) 939 and references therein.
12. Valterová, I., Unelius, R., Vrkoč, J. and Norin, T. *Phytochemistry. In press.*
13. Valterová, I., Lindström, M., Vrkoč, J. and Norin, T. *Naturwissenschaften. In press.*
14. Lindström, M., Roeraade, J. and Norin, T. *J. Chromatogr. 513* (1990) 315.
15. Hanessian, S. *Total Synthesis of Natural Products: The "Chiron" Approach.* Pergamon Press, Oxford 1983.
16. Novák, V., Hrozinka, F. and Starý, B. *Atlas of Insects Harmful to Forest Trees*, Elsevier, New York 1976, Vol. 1.
17. Borg-Karlson, A.-K. and Persson, M. *Phytochemistry. Submitted.*
18. Nordlander, G. *Entomol. Exp. Appl. 59* (1991) 229.
19. Nordlander, G. *J. Chem. Ecol. 16* (1990) 1307.

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