Journal of Chemical Ecology, Vol. 15, No. 2, 1989

VARIATION OF ENANTIOMERIC COMPOSITION OF α-PINENE IN NORWAY SPRUCE, *Picea abies*, AND ITS INFLUENCE ON PRODUCTION OF VERBENOL ISOMERS BY *Ips typographus*[1] IN THE FIELD[2]

MIKAEL LINDSTRÖM,[3] TORBJÖRN NORIN,[3]
GÖRAN BIRGERSSON,[4] and FREDRIK SCHLYTER[5]

[3]*Department of Organic Chemistry, Royal Institute of Technology*
S-100 44 Stockholm, Sweden

[4]*Department of Chemical Ecology, Göteborg University*
Box 33031, S-400 33 Göteborg, Sweden

[5]*Department of Animal Ecology, Lund University*
Ecology Building, S-223 62 Lund, Sweden

(Received August 3, 1987; accepted January 19, 1988)

Abstract—The enantiomeric composition of α-pinene in individual Norway spruce trees [*Picea abies* (L.) Karst.] was determined on a chiral GC column after stereoselective hydroboration–oxidation followed by a reaction with isopropyl isocyanate to form the carbamate derivative. The enantiomeric composition varied considerably between trees of different genetic origin. There was a strong correlation between the chirality of α-pinene in host spruce trees and the *cis/trans* ratio of verbenols found in the hindguts of the bark beetle *Ips typographus* (L.) infesting the trees.

Key Words—Enantiomeric composition, chiral separation, GC, pheromone, plant-insect relations, Norway spruce, *Picea abies*, α-pinene enantiomers, *cis*-verbenol, *trans*-verbenol, *Ips. typographus,* Coleoptera, Scolytdae.

INTRODUCTION

Many bark beetles in the genus *Ips* are known to produce *cis*- and *trans*-verbenol when they are exposed to the vapors of α-pinene or oleoresins from their

---

[1] Coleoptera; Scolytidae.
[2] Parts of this work have been presented at the 14th International Symposium on the Chemistry of Natural Products in Poznan, Poland, July 9–14, 1984.

host trees (Renwick et al., 1976; Byers, 1981). This production, which is a stereospecific allylic hydroxylation, gives (1*S*, 4*S*, 5*S*)-*cis*-verbenol from (−)-1*S*, 5*S*)-α-pinene, whereas (1*R*, 4*S*, 5*S*)-*trans*-verbenol is produced from the (+)-(1*R*, 5*R*)-enantiomer of α-pinene. Moreover, males of *I. typographus* produce *cis*- and *trans*-verbenol in ratios that were found to reflect the enantiomeric composition of the α-pinene, to which they were exposed in a laboratory experiment (Klimetzek and Francke, 1980). (*S*)-*cis*-Verbenol is also identified as a component of the aggregation pheromones of both *I. paraconfusus* (Silverstein et al., 1966) and *I. typographus* (Krawielitzki et al., 1977), while the attraction to *trans*-verbenol is small or uncertain (Dickens, 1981; Schlyter et al., 1987).

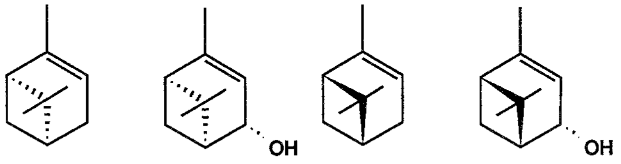
It is well known that there are genetic and seasonal variations of the monoterpene compositions of conifers (Juvonen, 1966; Rudloff, 1967). However, very little is known about the enantiomeric compositions due to the lack of convenient analytical methods.

α-Pinene is produced in different enantiomeric compositions by various conifer species (Klimetzek and Francke, 1980). Even within the same genus, i.e., *Pinus*, different species are known to vary from almost pure (+)- to almost pure (−)-enantiomer; α-pinene from *P. halipensis* exhibits $[\alpha]_D = +48.30°$, whereas that of *P. pinaster* (French turpentine) has $[\alpha]_D = -43.4°$ (Mirov, 1961). Pure (+)-α-pinene has been reported to have $[\alpha]_D = +52.4°$ (Comyns and Lucas, 1957). The intraspecific variation in the enantiomeric composition of monoterpene hydrocarbons, such as α-pinene, has to our knowledge not been investigated.

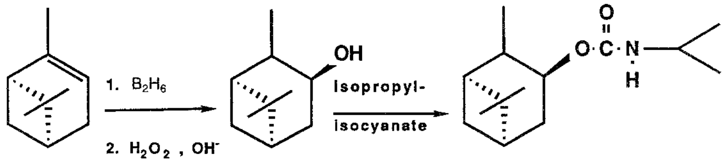
The methods available for the determination of the enantiomeric compositions of hydrocarbons are limited. The classical polarimetric method and NMR techniques require comparatively large amounts of pure samples and are not suitable for complex mixtures, whereas chromatographic techniques require relatively small sample amounts, and crude samples of complex mixtures can be analyzed.

Several chiral GC phases are known, and it is also possible to separate enantiomers as diastereomers on ordinary GC phases, but none of these techniques is general or works well for the resolution of monoterpene hydrocarbon enantiomers. The aim of the present investigation was to develop a sensitive chromatographic method for the determination of the enantiomeric composition of α-pinene in biological samples. Recently, methods for gas-chromatographic determinations of the enantiomeric compositions of secondary alcohols have been reported. The optical purity can be determined either via separation of diastereomeric derivatives [N-TFA-(L)-alanine esters] (Kruse et al., 1979) or by separation of isopropyl carbamates on a chiral GC phase, Chirasil-Val (König et al., 1982). There are also some reports on direct separation of alcohol enantiomers using chiral metal complexes as GC phase (Schurig et al., 1985).

It is possible to convert olefinic hydrocarbons regio- and stereoselectively into secondary alcohols via a hydroboration–oxidation reaction (Zweifel and Brown, 1964). We have applied this reaction to convert α-pinene into isopinocampheol. For this purpose we have used the two-phase technique of Brändström et al. (1972). The isopinocampheol was separated as its isopropyl carbamate according to the method of König et al. (1982) (Scheme 1).



(-)-[1S,5S]- -α-Pinene   [1S,4S,5S]-cis- Verbenol   (+)-[1R,5R]- -α-Pinene   [1R,4S,5R]-trans- Verbenol



(-)-α-Pinene   (-)-Isopinocampheol   Isopropyl carbamate

SCHEME 1.

We have used this method to study the enantiomeric composition of α-pinene in individual spruce trees, *Picea abies*, and compared it to the *cis/trans*-isomer ratio of verbenol found in the hindguts of male bark beetles, *Ips typographus*, attacking some of these trees.

METHODS AND MATERIALS

Biological Material. Phloem samples were taken from 29 different individual spruce trees [*Picea abies* (L) Karst.] at three different localities. The first was a forest plantation close to Uppsala, 70 km north of Stockholm. Samples were taken from 21 different 10-year-old cloned trees originating from Finland (SF) and West Germany (BRD). These samples were taken in February 1983 and stored as logs for six to eight months in individual polyethene bags at −18°C. Two spruce trees close to the laboratory in Stockholm made up a second group. The third source of phloem samples were six trees infested by spruce bark beetles, *Ips typographus*, sampled close to Torsby, province of Värmland, western Sweden, during the main swarming period in early June 1982 (cf. Birgersson et al., 1988). These phloem samples were removed with a knife and stored in liquid nitrogen until extraction and analysis.

From each spruce tree in the third group, male bark beetles were also collected and analyzed for their hindgut volatiles as described in detail by Birgersson et al. (1984).

Sample Preparation. The outer cork bark was removed with a knife, and the phloem sample was removed from the log with a rasp. On the infested trees the stored phloem sample was chopped with a knife. The phloem sample (1 g) was extracted with pentane (Merck p.a.) for 15 min in an ultrasonic bath. The pentane extract was washed with saturated sodium bicarbonate and passed through a short alumina column (2 g Merck, Aktiv 60, base treated) to remove chlorophyll and polar compounds. Most of the pentane was evaporated and dry dichloromethane (1 ml) was added. The solution was transferred to a 3-ml reaction vial equipped with a PTFE-coated septum. Tetrabutylammonium boronate (Brändström et al., 1972) (50 mg) was added. The vial was flushed with argon. Ethyl bromide (50 μl) was added with a syringe, and the reaction mixture was kept at 60°C for 30 min. A few drops of water were added to destroy the excess of hydride. Aqueous sodium hydroxide (2 N, 100 μl) was added, followed by a slow addition of hydrogen peroxide (35%, 70 μl). The vial was left at room temperature for 1 hr. Most of the dichloromethane was evaporated, and water and pentane were added. The pentane phase was separated and concentrated.

The concentrated pentane extract was dissolved in dichloromethane (200 μl), and isopropyl isocyanate (100 μl) was added. The mixture was heated to 100°C for 20 min. Dichloromethane and the excess of isopropyl isocyanate were evaporated and the residue dissolved in pentane and analyzed on GC.

The gas chromatographic analyses were performed on a PYE 204 GC and on a Hewlett-Packard 5830 GC both equipped with fused silica columns coated with XE-60-(*S*)-valine-(*S*)-α-phenylethylamide (Chrompac 50 m, ID 0.23, $df = 0.14$ mm, HETP = 0.21 mm). The oven was kept at 165°C isodermal, and injector and detector temperatures were 250°C. Helium was used as carrier gas (16–22 cm/sec). The α-factor for the two enantiomers was 1.0178.

Preparation of Test Mixtures and Isopinocampheol References. A test sample containing 60.0% of (−)-α-pinene was prepared by mixing (+)- and (−)-α-pinene isolated earlier at the Department of Organic Chemistry, Royal Institute of Technology (0.045 g of $[\alpha]_D = 19.6°$ and 0.053 g of $[\alpha]_D = -36°$) and used to check the steric course of the hydroboration step. Small samples (25, 50, 100, and 150 μg) were taken from the test mixture and hydroborated as described for the pentane extracts of spruce trees.

Isopinocampheol. Ethyl bromide (3.27 g) was slowly added to a solution of tetrabutylammonium boronate (2.57 g) and α-pinene (2.72 g) of high optical purity (Fluka) in dry dichloromethane (10 ml) under an atmosphere of argon. The mixture was refluxed for 30 min. The excess of hydride was destroyed with water. Aqueous sodium hydroxide (2 N, 5 ml) was added, followed by dropwise addition of hydrogen peroxide (35%, 3 ml). The mixture was stirred at room temperature for 1 hr. The dichloromethane phase was separated and dried, and the solvent was slowly evaporated to yield crude isopinocampheol, which was used as reference compound for the GC analysis.

RESULTS AND DISCUSSION

The α-pinene samples from the test mixture were analyzed and found to contain 60.9 ± 0.7% (min 59.9%, max 61.4%, $N = 6$) of the (−)-enantiomer, close to the expected 60.0%. The experiment shows that the hydroboration is stereoselective and that both accuracy and precision of this method for detecting the enantiomeric composition of α-pinene are good.

The enantiomeric composition of α-pinene in the phloem of Norway spruce trees was found to vary between 32 and 98% of the (−)-enantiomer (corresponding to 38% ee of (+)-α-pinene and 96% ee of (−)-α-pinene) (Table 1).

Males of the spruce bark beetle (*Ips typographus*) were collected from six infested Norway spruce trees and the hindguts were extracted and analyzed for the total amount of *cis*-verbenol (Birgersson et al., 1988) and the ratio of *cis*- to *trans*-verbenol. The relative amounts of verbenol isomers correlated strongly ($r = 0.91$, $P < 2\%$) to the relative amounts of (−)- and (+)-α-pinene in the phloem (Figure 1). This result is in accordance with the laboratory experiments performed by Klimetzek and Francke (1980).

TABLE 1. PERCENTAGE OF (+)-ENANTIOMER OF TOTAL AMOUNT OF α-PINENE IN NORWAY SPRUCE

	N^a	$\bar{X}$ ± S.D.	Min	Max
Trees BRD[b]	11	12.9 ± 9.9	1.9	30.0
Trees SF[c]	10	36.9 ± 11.2	15.0	51.3
Infested	6	31.3 ± 23.0	5.0	61.0
Trees Sthlm[d]	2		13.0	67.9
All trees	29	27.5 ± 18.9	1.9	67.9

[a] Numbers of trees investigated.
[b] Trees originating from three different clones from W. Germany.
[c] Trees originating from three different clones from Finland
[d] Trees from Stockholm.

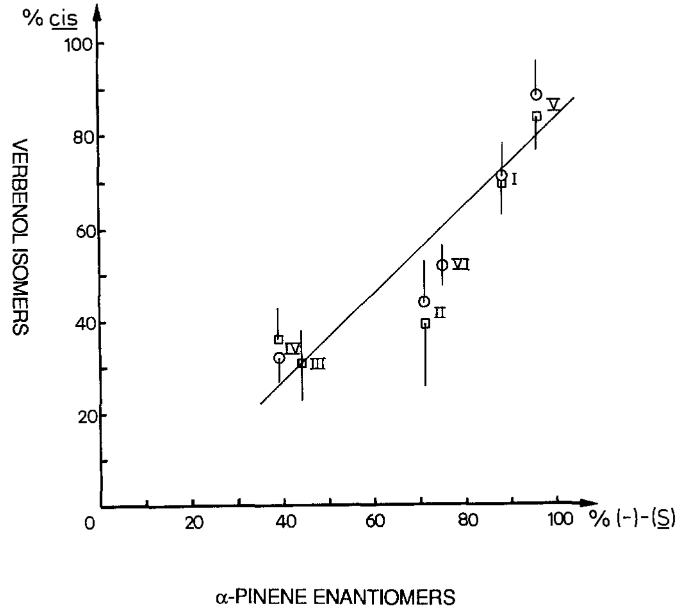


FIG. 1. Percentage *cis* isomer of verbenol from *Ips typographus* male hindguts versus percentage (−)-enantiomer of α-pinene found in *Picea abies* phloem extracts from infested trees (I–VI). □ = attack phase 3, $\bar{X} \pm$ SD; ○ = attack phase 6, $\bar{X} \pm$ SD, attack phases according to Birgersson et al. (1984). ($N = 10$; $k = 0.91$; $m = 9.23$ ($y = kx + m$); $r = 0.91$; $r^2 = 0.82$.) In tree III, no beetles in attack phase 6 were found, and in tree VI no beetles in attack phase 3 were found.

The intraspecific variation in the enantiomeric composition of α-pinene in spruce was probably the reason for the findings by Birgersson et al. (1984) that batch samples of *I. typographus* males attacking different spruce trees varied in their ratios of *cis*- and *trans*-verbenols.

Since *cis*-verbenol is an essential component of the aggregation pheromone of *Ips typographus* and as there is a direct relation between the *cis* ratio of verbenols found in the hindguts of the bark beetle and the enantiomeric composition of α-pinene in the phloem of the host tree, we assume that there also exists a relationship between the enantiomeric composition in the Norway spruce and its resistance to attacks of *Ips typographus*. Trees with a very low content of (−)-α-pinene may thus be resistant to bark beetle attacks. Further studies to test this hypothesis are in progress.

There is also a strong indication that the enantiomeric composition within a clone is constant. However, the amount of material investigated is too small to settle this point. Work is in progress to study the inheritance of the enantiomeric composition of α-pinene.

During the course of this investigation, a new method using α-cyclodextrin and formamide as the stationary phase in gas chromatography for the separation of α-pinene has been published (Koscielski et al., 1983). This method has been shown to be very useful for the enantiomeric separation of several of the common monoterpene hydrocarbons (Lindström et al., 1987). However, further development is needed before this method can be applied to complex mixtures.

Acknowledgments—We thank Åke Lindelöw, The Swedish University of Agricultural Sciences, Uppsala, for supplying us with cloned trees. Ann-Britt Wassgren is gratefully acknowledged for information regarding chiral GC columns. This study was made within the Swedish project "Odour Signals for Control of Pest Insects," funded by the Swedish Research Councils (NFR, FRN, SJFR and STU), Nils and Dorothy Troedssons Forskningsfond, and Swedish forest industries.

REFERENCES

BIRGERSSON, G., SCHLYTER, F., LÖFQVIST, J., and BERGSTRÖM, G. 1984. Quantitative variation of pheromone components in spruce bark beetle *Ips typographus* from different attack phases. *J. Chem. Ecol.* 10:1029–1055.

BIRGERSSON, G., SCHLYTER, F., BERGSTRÖM, G., and LÖFQVIST, J. 1988. Individual variation in the aggregation pheromone content of the spruce bark beetle, *Ips typographus*. *J. Chem. Ecol.* Submitted.

BRÄNDSTRÖM, A., JUNGGREN, U., and LAMM, B. 1972. An improved method for the preparation of solutions of diborane. *Tetrahedron Lett.* 31:3173–3176.

BYERS, J.A. 1981. Pheromone biosynthesis in the bark beetle, *Ips paraconfusus*, during feeding or exposure to vapours of host plant precursors. *Insect Biochem.* 11:563–569.

COMYNS, A.E., and LUCAS, H.J. 1957. Coordination of silver ion with unsaturated compounds. IX. Solid complexes of silver salts with cyclohexene, α-pinene and β-pinene. *J. Am. Chem. Soc.* 79:4339–4341.

DICKENS, J.C. 1981. Behavioural and electrofysiological responses to the bark beetle, *Ips typographus*, to potential pheromone components. *Physiol. Entomol.* 6:251–261.

JUVONEN, S. 1966. Über die Terpenbiosynthese in *Pinus silvestris*. *Acta Bot. Fenn.* 71:5–92.

KLIMETZEK, D., and FRANCKE, W. 1980. Relationship between the enantiomeric composition of α-pinene in host trees and the production of verbenols in *Ips* species. *Experentia* 36:1343–1345.

KÖNIG, A.W., FRANCKE, W., and BENECKE, I. 1982. Gas chromatographic enantiomer separation of chiral alcohols. *J. Chromatogr.* 239:227–231.

KOSCIELSKI, T., SYBILSKA, D., and JURCZAK, J. 1983. Separation of α-pinene and β-pinene into enantiomers in gas–liquid chromatography systems via cyclodextrin inclusions complex. *J. Chromatogr.* 280:131–134.

KRAWIELITZKI, S., KLIMETZEK, D., BAKKE, A., VITÉ, J.P., and MORI, K. 1977. Field and laboratory responce of *Ips typographus* to optically pure pheromonal components. *Z. Angew. Entomol.* 83:300–302.

KRUSE, K., FRANCKE, W., and KÖNIG, W.A. 1979. Gas chromatographic separation of chiral alcohols, amino alcohols and amines. *J. Chromatogr.* 170:423–429.

LINDSTRÖM, M., NORIN, T., and ROERAADE, J. 1987. Gas chromatographic separation of monoterpene hydrocarbon enantiomers using α-cyclodextrin and formamide/water as stationary phase. *J. Chromatogr.* Submitted.

MIROV, N.T. 1961. Composition of gum turpentines of pines. U.S. Department of Agriculture, Technical bullentin No. 1239. ($[\alpha]_D$ values from Beilsteins Handbuch der Organischen Chemie, Vierte Auflage, 1922, Band 5, page 146.)

RENWICK, A.A., HUGHES, P.R., and KRULL, J.S. 1976. Selective production of *cis* and *trans*-verbenol from (−)-α- and (+)-β-pinene by a bark beetle. *Science* 191:199-201.

RUDLOFF, E. VON. 1967. Chemosystematic studies in the genus *Picea* (Pinaceae). I. Introduction. *Can. J. Bot.*, 45(6):891-901.

SCHLYTER, F., BIRGERSSON, G., BYERS, J.A., LÖFQVIST, J., and BERGSTRÖM, G. 1987. Field responce of the spruce bark beetle, *Ips typographus*, to aggregation pheromone candidates. *J. Chem. Ecol.* 13:701-716.

SCHURIG, V., LEYRER, U., and WEBER, R. 1985. The use of glass and fused silica open tubular columns for the separation of structural, configural and optical isomers by selective complexation GC: sec-alcohols and ketones. *HRC & CC* 8:459–464.

SILVERSTEIN, R.M., RODIN, J.O., and WOOD, D.L. 1966. Sex attractants in frass produced by male *Ips confusus* in Ponderosa pine. *Science* 154:509-510.

ZWEIFEL, G., and BROWN, H.C. 1964. Hydroboration of terpenes II. The hydroboration of α-pinene and β-pinene—the absolute configuration of the dialkyborane from the hydroboration of α-pinene. *J. Am. Chem. Soc.* 86:393–397.