d'autres auteurs[12–14] ont mesuré une diminution importante du taux de cytochrome P_{450} dans les microsomes hépatiques lorsque le cadmium est administré par voie i.p. en une seule dose. Au niveau des mitochondries du cortex rénal où la 1α-hydroxylation de la 25 hydroxyvitamine D_3 s'effectue, aucune différence dans les taux de cytochrome P_{450} entre animaux témoins et traités n'a pu être mise en évidence. Cependant Lorentzon[15] trouve que la conversion du 1,25 $(OH)_2D_3$ dans les reins après administration de 25 hydroxy 26, 27 méthyl ^3H cholécalciférol est très diminuée chez des rats femelles soumis à un régime normal en calcium et intoxiqués chroniquement par le cadmium per os. Kimura[16] montre in vivo que, chez des rats soumis pendant 3 semaines à un régime pauvre en calcium et vitamine D contenant 300 ppm de cadmium, cette réaction d'1α-hydroxylation du 25 (OH) D_3 s'effectue. En outre Suda[7] montre que, in vitro, l'addition de 0,025 mM de $CdCl_2$ inhibe complètement l'activité de la 25 (OH) D_3 1 hydroxylase dans les mitochondries de reins de poulets, mais que chez des rats soumis à un régime pauvre en vitamine D, l'hydroxylation du C_1 du 25 (OH) D_3 se faisait même si les animaux recevaient des quantités importantes de cadmium. Ces résultats conduisent les auteurs à envisager la protection possible vis à vis du cadmium par la "cadmium binding protein" dont la synthèse est induite lors de l'administration continue de cadmium[10,17,18] ce métal étant pratiquement sans effet sur les hydroxylases de la vitamine D_3. Conformément à divers travaux[19–23] un effet direct du cadmium sur la "calcium binding protein" apparaît dès lors vraisemblable.

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Relationship between the enantiomeric composition of α-pinene in host trees and the production of verbenols in *Ips* species[1]

D. Klimetzek and W. Francke

Forstzoologisches Institut der Universität, D-78 Freiburg/Br. (Federal Republic of Germany), and Institut für Organische Chemie der Universität, D-2 Hamburg (Federal Republic of Germany), 29 February 1980

Summary. Upon exposure to the vapours of oleoresin from 8 conifers, bark beetles *Ips typographus* and *I. amitinus* produced verbenol, a terpene alcohol, in a predictable pattern. Apparently, this pattern changed in relation to the varied enantiomeric composition of the α-pinene contained in the resin of the various coniferous species. For calibration, defined mixtures of (+)- and (−)-α-pinene were used to establish the different levels of beetle response in the production of cis- and trans-verbenol. Methodical and ecological implications of the phenomenon are discussed.

Derivatives of monoterpene hydrocarbons may act as important signals in the chemical communication systems of bark beetle species feeding in the phloem tissue of conifers[2]. For instance, several species of the genus *Ips* use 2-methyl-6-methylene-2,7-octadien-4-ol (ipsdienol) and 2,6,6-trimethylbicyclo[3.1.1]hept-2-en-4-ol (verbenol) as components of their aggregation pheromone[3].

Hughes[4] found ipsdienol in male *Ips paraconfusus* Lanier after exposure to vapours of 2-methyl-6-methylene-2,7-octadiene (myrcene), a host plant monoterpene. Also, quantitative relations between myrcene concentration and ipsdienol production have been reported recently[5].

I. paraconfusus selectively converts the enantiomers of 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (α-pinene) to diastereomeric isomers of verbenol[6] and the same phenomenon has since been observed in other species[7] suggesting the existence of oxidase systems generating hydroxyl groups in the allyl position of certain host terpenes. Whereas under natural conditions the oxygenation of myrcene appears to be restricted to males only, the occurrence of the verbenols does not seem to be sex specific.

The object of this study was to quantify the relationship which seems to exist between the enantiomeric ratios of the α-pinene contained in the oleoresin of the various conifers and the verbenols produced by the *Ips* beetles upon exposure to the resinous vapours. Our work, however, did not intend to investigate possible routes of verbenol biosynthesis as the verbenols may be produced by direct conversion of α-pinene or de novo upon pinene induction and, possibly, also by associated microorganisms[8].

Materials and methods. Beetles of *Ips typographus* (L.)

and *I. amitinus* (Eichh.) were collected as they emerged from naturally infested logs kept in rearing rooms at 18–20 °C. The beetles were held for 2–3 days under refrigeration at 4 °C until used.

20 beetles of each *Ips* species were placed into petri dishes containing plastic caps filled with 1 ml of a defined (+)-/(−)-α-pinene mixture[3]. Each sample was exposed to the vapours for 24 h in the dark. 6 different mixtures were used in 4 repetitions; untreated beetles served as control. Assuming that the highest rotation value reported for (+)-α-pinene[9] represents an optically pure sample, optical purity of the commercially available α-pinenes (Fluka GmbH) was 94% for the (+)-α-pinene and 88% for the (−)-isomer as determined by their optical rotation on a Perkin-Elmer 421 polarimeter at 589 nm. Chemical purity of the samples was greater than 99%.

In addition, *I. typographus* was exposed to (−)-β-pinene (Fluka GmbH) and defined mixtures of (+)- and (−)-α-pinene and (−)-β-pinene.

Oleoresin from different conifers (figure 2) was tapped from small holes drilled into the xylem[10], collected in amber glass vials and stored at −20 °C until used. For *T. canadensis* which does not contain resin ducts, oleoresin was collected from cortical blisters. The beetles were exposed to the vapours of 1 g oleoresin in the same manner as described for the α-pinene treatment.

After exposure, the beetles were dissected and their sex determined. The cis: trans-ratio of verbenol was determined for each sex by headspace GLC using a 3 m, 2 mm inner diameter glasscolumn packed with FFAP on chromosorb W-AW DMCS at 140 °C or from pentane extracts of crushed beetles on a 50-m glass capillary column coated with WG 11 at 100 °C. The chirality of the verbenols contained in the beetles after exposure to α-pinene resp. oleoresin was determined by methods described elsewhere[11].

α-Pinene from conifers was separated from the low boiling fraction of oleoresin by preparative GLC on a 2 m, 8 mm inner diameter column with FFAP as stationary phase under a temperature programme from 120–160 °C at a rate of 4 °C/min. The enantiomeric α-pinene composition was determined by measuring the optical rotation of the mixture in pentane at 21 °C using the commercially available products as internal standards.

Results and discussion. The gas chromatographic and mass spectroscopic data for the N-trifluoracetyl-L-alanylesters from verbenols found in the beetles indicate that both *Ips* species preferentially produce (−)-(1S,4S,5S)-cis-verbenol and (+)-(1R,4S,5R)-trans-verbenol, respectively, in response to (−)-(1S,5S)- and (+)-(1R,5R)-α-pinene[12]. Smaller amounts of (−)-(1S,4R,5S)-trans-verbenol and (+)-(1R,4R,5R)-cis-verbenol are produced as byproducts. This suggests that chiral oxidase systems are functioning, which introduce a hydroxy group at C_4 in α-pinene, stereoselectively generating a (4S)-configuration (figure 1).

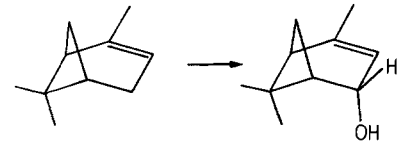
Males of *I. typographus* and both sexes of *I. amitinus* produced appreciable amounts of the verbenols upon exposure to the various (+)-/(−)-α-pinene mixtures while the verbenol concentration found in *I. typographus* females was relatively low. By comparison, the verbenol content in non-exposed beetles was negligible. Production of both verbenols was 3 times higher in *I. amitinus* (♂ : ♀ = 1.6 : 1) than in *I. typographus* (♂ : ♀ = 4.7 : 1). The ratio of cis: trans-verbenol showed a linear increase with an increasing proportion of (−)-α-pinene (figure 2). Exposure to 94% (+)-α-pinene led to about 10% cis-verbenol while 88% (−)-α-pinene yielded about 70% of cis-verbenol indicating 95% stereoselectivity of the enzymatic oxygenation of (+)-α-pinene and 80% of (−)-α-pinene. Results agreed well for both species and sexes and in different runs; in figure 2, deviations are indicated.

After exposure to oleoresin, the beetles produced about 15% of the verbenol quantity in comparison to the treatment with the pure α-pinene. Obviously, this is due to the lower α-pinene concentrations in the various oleoresins. On average, the total verbenol production induced by resin exposure was 6 times higher in *I. amitinus* (♂ : ♀ = 1.1 : 1) than in *I. typographus* (♂ : ♀ = 4.9 : 1).

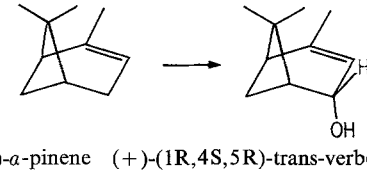
Under the assumption that α-pinene represents the only stimulus of verbenol production in these *Ips* species, the cis: trans-ratio found in beetles exposed to oleoresin should match a 'calibration curve' as shown in figure 2. In fact, the data obtained from the oleoresin-exposed beetles fit the calibration curve reasonably well. However, the amounts of cis-verbenol produced were somewhat higher in some cases than estimated from the apparant (−)-α-pinene content. This may suggest that the beetles are capable of using additional host compounds for the production of the verbenols.

Similar to *Dendroctonus* spp.[13], *I. typographus* seems to allyloxygenate (−)-2-methylene-6,6-dimethylbicyclo[3.3.1]-heptane ((−)-β-pinene) to trans-2-methylene-6,6-dimethylbicyclo [3.3.1]heptane-3-ol (trans-pinocarveol). Exposures of *I. typographus* to 1:1 mixtures of (−)-α- and (−)-β-pinene led to a ratio of 3–5:1 of cis-verbenol and trans-pinocarvenol, respectively.

I. typographus uses (4S)-cis-verbenol as a major phero-



(−)-(1S,5S)-α-pinene (−)-(1S,4S,5S)-cis-verbenol



(+)-(1R,5R)-α-pinene (+)-(1R,4S,5R)-trans-verbenol

Fig. 1. Configuration of α-pinene enantiomers and resulting verbenols.

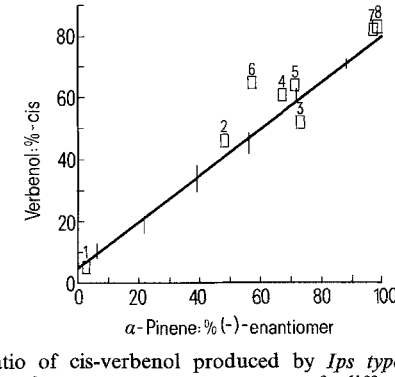


Fig. 2. Ratio of cis-verbenol produced by *Ips typographus* and *I. amitinus* after exposure to vapours of different synthetic (+)-/(−)-α-pinene mixtures (vertical bars) and oleoresins of 8 conifer species (rectangles): 1: *Pinus insularis* E.; 2: *Pinus silvestris* L.; 3: *Pinus strobus* L.; 4: *Larix leptolepis* G.; 5: *Picea abies* (L.); 6: *Larix decidua* M.; 7: *Tsuga canadensis* C.; 8: *Pseudotsuga taxifolia* P.

monal component signalling aggregation and host colonisation. The obvious dependence of (4S)-cis-verbenol production on the chemical makeup of a host tree's oleoresin, and particularly the enantiomeric composition of the α-pinene contained therein, suggests a unique feed-back system in the plant/insect relationship. Potentially, a host tree supports the insect pest's aggregation system to the extent to which the tree's oleoresin contains (−)-α-pinene. Besides such ecological implications, the experimental procedure described may serve as a simple method in the gross examination of resinous materials as to their enantiomeric composition of α-pinene.

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Reduction of ferricytochrome c by human red cells

A. Tomoda, M. Ida, Y. Yoneyama, S. Kitajima and S. Minakami

Department of Biochemistry, Kanazawa University, School of Medicine, Kanazawa 920 (Japan), and Department of Biochemistry, Faculty of Medicine, Kyushu University, Fukuoka 813 (Japan), 26 February 1980

Summary. Human red cells reduced extracellular ferricytochrome c to ferrocytochrome c under various conditions, suggesting that ferricytochrome c reducing systems are present at the outer surface of the red cell membrane.

Though it has been indicated that there are some electron transfer systems in the human red cell membrane, little is known about their nature. Mishra and Passow[1] observed that the ferricyanide anion, which does not penetrate into red cells, is reduced by human red cells. They considered that this phenomenon is probably correlated with intracellular metabolism, because the reduction of ferricyanide decreased under conditions in which red cell glycolysis is depressed. Recently Orringer and Roer[2] proposed that ferricyanide may be reduced by ascorbate-mediated transmembrane reducing systems of the cell membrane. In spite of these reports, the possibility that the reducing systems are present in the outer membrane of the red cells is not discussed.

In the work described in this paper, we investigated whether or not ferricytochrome c, which also does not penetrate into red cells (own unpublished data), can be reduced by whole human red cells. We found that ferricytochrome c was reduced significantly by human red cells, and that the reduction was correlated neither with intracellular metabolism nor with ascrobate-mediated transmembrane reducing systems of the cell membrane. Our results suggested that ferricytochrome c reducing systems are present on the outer surface of the red cell membrane.

Methods. Red cells were obtained from 1-day-old ACD blood after centrifugation at 3000 rev./min for 10 min. After removal of buffy coats and plasma, the red cells were washed with about 5 vol. of 0.9% NaCl solution 5 times. By this procedure, leucocytes and thrombocytes were almost completely removed, and glucose-free red cells were obtained. Then the red cells were suspended in Krebs-Ringer solution containing 120 mM NaCl, 2 mM potassium phosphate, 1 mM $MgCl_2$, 5 mM KCl (hematocrit value; 30%). The pH of the suspension was adjusted to 7.4 by the addition of 0.1 M NaOH in Krebs-Ringer solution at 37 °C. The red cell suspensions were used for the following experiments. 1. Either NADH, NADPH (final concentrations; 1 mM), NAD, NADP (5 mM), FMN (1 mM), superoxide dismutase (116 units) or ascorbate oxidase (14.7 units) was added to 25 ml of red cell suspensions, which were incubated at 37 °C after the addition of glucose (10 mM). The reaction was initiated by the addition of ferricytochrome c solutions (200 μM). 5 ml of the samples were taken out at intervals for analysis, and centrifuged immediately at 10,000 rev./min for 1 min. There was no hemolysis due to these treatments. The supernatants were diluted with 4 vol. of Krebs-Ringer solution, and the absorption spectra of the supernatants were measured between 450 and 650 nm spectrophotometrically. 2. The experimental conditions are the same as those stated in (1), except that 1 mM deoxyglucose was added in place of glucose. 3. 2 mM NEM or PCMBS was added to the red cell suspensions, which were further held at 37 °C for 5 min. Free NEM and PCMBS were removed from the suspension by washing with Krebs-Ringer solutions (centrifuged at 8000 rev./min for 1 min twice). The red cells obtained by this procedure were resuspended in a Krebs-Ringer solution to make at 30% hematocrit. After adjustment of the pH of the suspension to 7.4 with 0.1 M NaOH dissolved in Krebs-Ringer solution, the reaction was initiated by the addition of ferricytochrome c solution. The absorption spectrum of the supernatant was measured.

Results. Figure 1, A shows the absorption spectra of the ferricytochrome c containing supernatants of the samples at 0-90 min (control) between 450 and 650 nm. The absorbance at 521 and 550 nm increased with time, and the isosbestic points were clearly observed at 502, 526, 542 and 556 nm. The addition of NADH, NADPH, NAD, NADP,