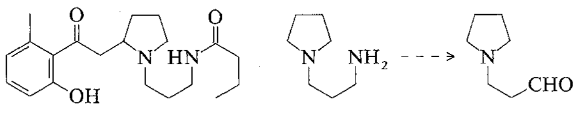
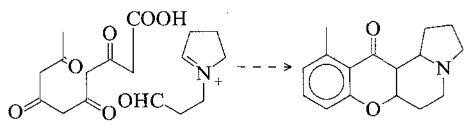
Dinorperipentadenine (**5**)[7] is a homologue of (**4**) with a shorter side-chain, which is evidently formed in a similar way to (**4**) from butanoic instead of hexanoic acid.



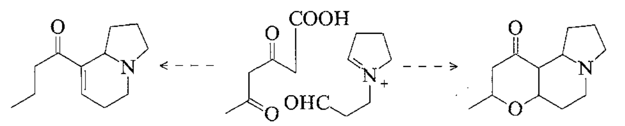
5 Dinorperipentadenine **3** **6**

The diamine (**3**) could be metabolized by oxidative deamination into the aminoaldehyde (**6**), which could then serve as a building block for the biogenesis of elaeocarpidine (**1**), elaeocarpine (**7**) and other C16 *Elaeocarpus* alkaloids[4]:



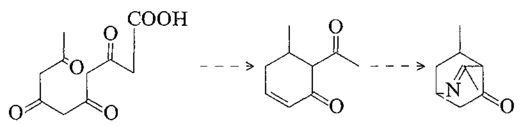
7 Elaeocarpine

The C12-type bases such as elaeokanines A (**8**) and D (**9**) could be derived in a similar way from a shorter polyketide chain:



8 Elaeokanine A **9** Elaeokanine D

The *Peripentadenia* alkaloid mearsine (**10**)[8] is exceptional inasmuch as acetate units appear to form the only source of carbon atoms in its structure:



10 Mearsine

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(+)-α-Pinene in the defensive secretion of *Nasutitermes princeps* (Isoptera, Termitidae)

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Summary. The frontal secretion of *Nasutitermes princeps* consists of 89% diterpenes and 11% monoterpenes. In the samples studied, (+)-α-pinene, whose optical purity reaches 99.5%, accounts for more than 80% of the monoterpenic content.
Key words. (+)-α-Pinene; *Nasutitermes princeps*; defensive secretion.

One of the most obvious characteristics of the genus *Nasutitermes* (Isoptera, Termitidae, Nasutitermitinae) is the existence of a highly specialized defensive caste. Their defensive mechanism consists of the ejection of a gluey frontal secretion onto predators or competitors which are thereby quickly incapacitated. The secretion consists mainly of a mixture of membrane-derived diterpenes dissolved in monoterpenes[4-7].

Besides being a solvent for the diterpenes, the monoterpenes have a role as alarm pheromones, which has been demonstrated in some termites[8-11], and are also irritant[10] and toxic[12-14]. Diterpenes are responsible for secretion viscosity, and as solutes may reduce monoterpene evaporation[4].

The nature of the monoterpenoid components has been studied in some Nasutitermitinae species reviewed by

Everaerts et al.[14]. Until now, the absolute configuration of the monoterpenic constituents has never been elucidated, although enantiomeric composition is of prime importance in the case of bioactive compounds[15].

Nasutitermes princeps (DESNEUX) (Isoptera, Termitidae) offers the possibility of investigating the absolute configuration of its major monoterpenic component. The structure of each of the components in the two fractions of its defensive secretion is well-known[7,14,16,17], and α-pinene represents more than 80% of the monoterpenic fraction of this secretion in the samples analysed; all the other monoterpenic constituents are present in low or trace amounts[14].

10,000 soldiers of *N. princeps*, collected from a nest in Bogia district (Madang Prov., Papua New Guinea), were extracted with 20 ml of hexane. This extract was evaporated under nitrogen down to 4 ml, and then analyzed by gas chromatography (GC). Identification of α-pinene was based on Kovats' indices at 80 °C and 90 °C, and confirmed by mass spectrometry (GC-MS). Mass spectra and Kovat's indices were compared with published data[14,18].

The separation of α-pinene from other compounds was performed on a Sep-Pak silica column from Waters Assoc. (Milford, MA, USA). Aliquots (500 µl) of the extract were eluted successively with 1.25 ml of dichloromethane (rate: 350 µl/min, fraction F1), 1 ml of dichloromethane (rate: 3 ml/min, fraction F2), 4 ml of the same solvent (rate: 24 ml/min, fraction F3), 3 ml of methanol (rate: 24 ml/min, fraction F4), and finally again 4 ml of dichloromethane (rate: 24 ml/min, fraction F5). Each fraction obtained from an aliquot was separately collected and then combined with the corresponding fractions from the other aliquots. These pooled fractions were evaporated under a nitrogen stream to obtain 5 fractions of 2 ml. Each fraction was then analyzed by GC as described above, and the chromatogram compared with the chromatogram of the total extract to determine the content of mono- and diterpenes. The total quantity of α-pinene collected was estimated by GC analysis using an external standard[19]. 1 ml of the final F1 fraction was examined on a Roussel-Jouan Quick Polarimeter (20 °C, optical path of the cell: 5 mm) to determine its rotation value. For comparison, we measured standard solutions of commercially available α-pinene[20] at the same concentration. Two wavelengths were used for these measurements, i.e. 546 nm and 589 nm (D-ray) (the results are given with their calculated absolute error[21]). Enantiomeric composition was further confirmed by gas chromatography using modified cyclodextrin as stationary phase[22,23].

The crude extract demonstrated the characteristic pattern of *Nasutitermes princeps* frontal secretion. The corresponding chromatogram is given in figure 1. In the monoterpenic fraction, α-pinene is strongly prominent (96% of this type of compound); the other monoterpenic constitutents (limonene, sabinene and terpinolene) are only present in trace amounts. Monoterpenes represent 11% of the total secretion. After the first separation, almost half of the α-pinene was recovered in the F1 fraction: the concentration of α-pinene in the F1 fraction was 3.1 mg/ml in dichloromethane, and its purity was

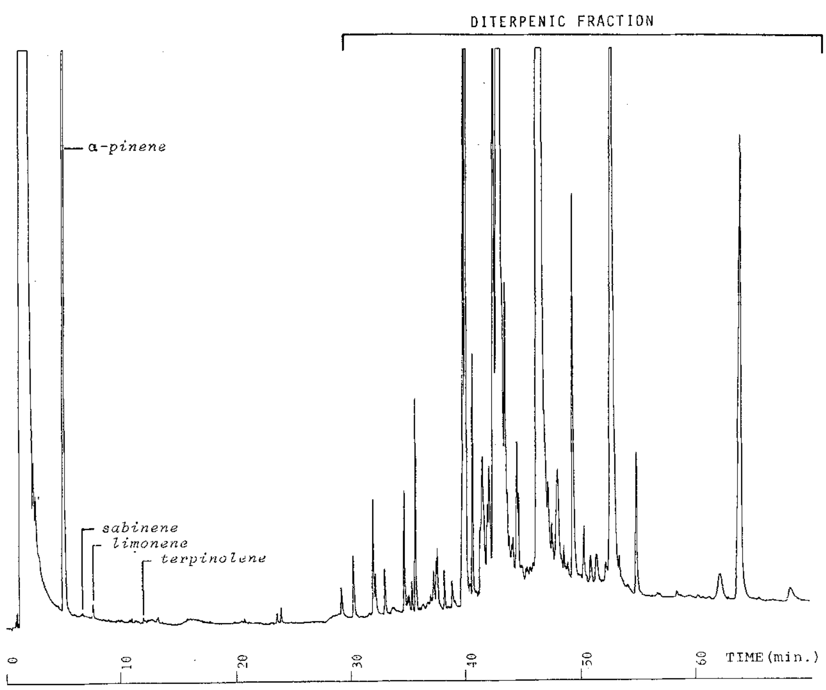

Figure 1. Gas chromatogram of the defensive secretion of *N. princeps*. GC conditions: silica capillary column DB-5, 30 m; from 50 °C to 280 °C (rise: 4 °C/min); 0.7 bar He.

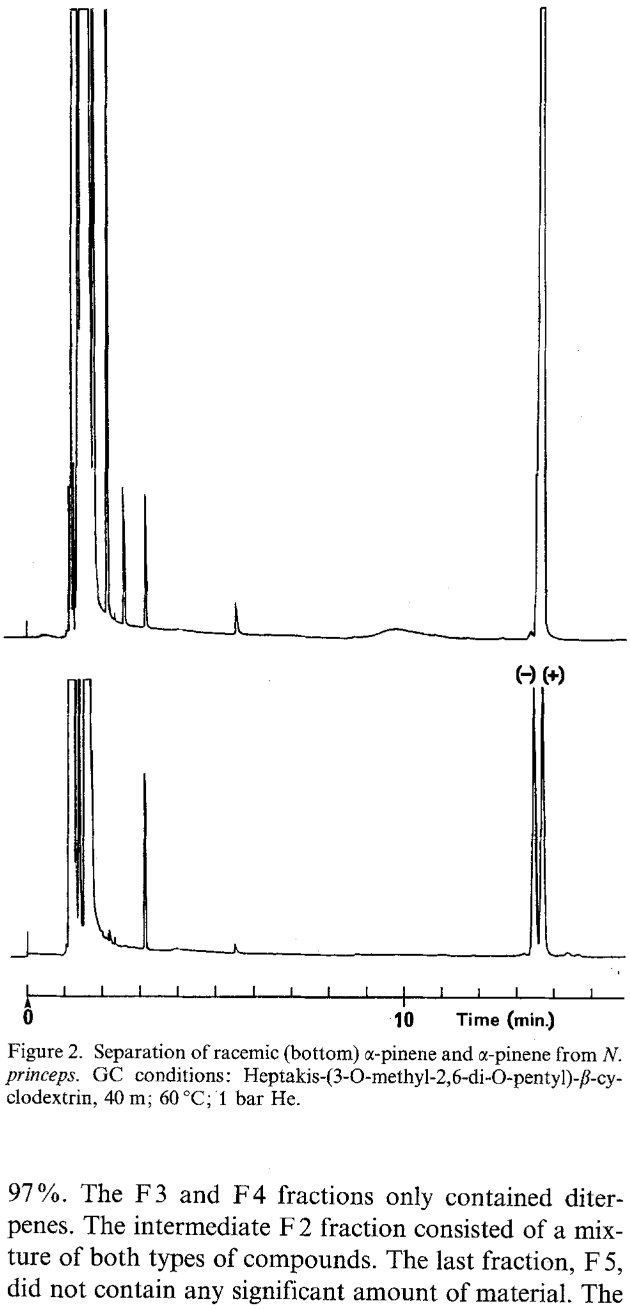

Figure 2. Separation of racemic (bottom) α-pinene and α-pinene from *N. princeps*. GC conditions: Heptakis-(3-O-methyl-2,6-di-O-pentyl)-β-cyclodextrin, 40 m; 60 °C; 1 bar He.

97%. The F3 and F4 fractions only contained diterpenes. The intermediate F2 fraction consisted of a mixture of both types of compounds. The last fraction, F5, did not contain any significant amount of material. The F2 fraction was reprocessed in the same way, to give 4.5 mg of additional α-pinene (purity: 97%). The impurities were traces (< 1%) of terpinolene, limonene and sabinene. Among these, only the last two are chiral and could interfere with the following measurements. For the F1 fraction, the rotatory power at 589 nm was $[\alpha]_D^{20} = +52°$ (± 4) (c = 0.38, dichloromethane), and at 546 nm, $[\alpha]_{546}^{20} = +75°$ (± 3) (c = 0.38, dichloromethane). These values were almost identical to those measured for the solution of the authentic sample (+)-α-pinene which were respectively, + 51° (± 3) (c = 0.38, dichloromethane) and + 77° (± 3) (c = 0.38, dichloromethane).

Gas chromatographic investigation of the F1 fraction on the chiral column revealed an optical purity of (+)-α-pinene of at least 99.5% (fig. 2).

There are many recorded examples of significant differences in biological activity between the enantiomers of chiral enantiomeric semiochemicals in ants, sawflies, beetles and butterflies[15]. These differences may lead to effective reproductive isolation, achieved between sympatric species through pheromone communication utilizing different enantiomeric compositions of the same semiochemical[24,25]. The species specificity in pheromone response could be due to the existence of two types of receptor cells, each specialized for one of the enantiomers, as demonstrated by Mustaparta et al.[26] with two species of bark beetle. The studies published so far have always been concerned with pheromones, and very little is known about chiral defensive compounds. Though there is a lack of data about this, it might be expected that the sites of molecular interactions involved in toxic action of defensive components would be chiral, and some authors have pointed to the importance of determining the absolute configuration of chiral defensive components[27].

α-Pinene is present in the frontal secretion of *N. princeps* in almost pure (+)-form; this enantioselectivity in the biosynthesis is not surprising since the enzymatic systems involved in the biosynthesis of each enantiomer of α-pinene are different[28,29]. However, in plants α-pinene occurs naturally as both (+) and (−) compounds, with a wide range of optical purity nearly always different from 100%[30].

The possible function of this particular enantiomer of α-pinene in the defense secretion of *N. princeps* is not fully understood. The toxic, repellent or irritant properties[10,12–14,31] of monoterpenes do not seem to need a determined enantiomeric composition, but such a composition could be correlated to a pheromonal role. This possibility is currently being investigated in our laboratories.

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Announcements

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