(*E,E*)-α-Farnesene the Main Substance of the Volatiles of the Flowers from European Mistletoe (*Viscum album* L.)

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(*E,E*)-α-Farnesene was extracted as the main component of the volatile fraction of male *Viscum album* L. Male and female flowers of *V. album* L. growing on different host trees were analysed by solid phase microextraction and by lipophilic extraction.

Introduction

The European mistletoe is a winter green hemiparasitic plant that parasitizes various trees. Three different subspecies exist according to host tree specificity:

V. album L. ssp. *album* L. growing on hardwood trees;

V. album L. ssp. a*bietes* Beck, growing on *Abies* sp.

V. album L. ssp. *laxum* Fick (ssp. *austriacum* Wiesb. Vollmann) growing on pine trees and very rarely on spruce. The plant is dioecous with small reduced flowers which open dependent on climate, between late February and early April (Luther and Becker, 1987). Despite their small size and their insignificant appearance they are pollinated by insects (Wallden, 1961). The flowers exhibit a fruit-like odour. It was suggested earlier from GC/MS-analysis that this odour is due to a sesquiterpene hydrocarbon with a molecular mass of 204 (Luther and Becker, 1987).

In the present work, we have isolated the main component of the volatile fraction together with a minor substance. The pure compounds were taken as external standards to analyse male and female flowers from different host trees.

Experimental

Plant material

Terminal short stems (ca. 5 mm) with male or female flowers were collected from mistletoes growing on different host trees. For preparative isolation a large quantity of male flowers were collected from mistletoes growing on apple trees near Hermersdorf, Saarland/Germany.

Isolation of α-(E,E)-farnesene

3.0 kg of male mistletoe flowers were extracted with 6 l dichloromethane and the solution left at –20 °C overnight. A sediment formed and after filtration the solution was dried with sodium sulfate. The solution was taken to dryness, dissolved in a small volume of dichloromethane and subjected to vacuum liquid chromatography (VLC) on silica gel (15 μm). The column was eluted with 500 ml of pentane. After evaporation of the pentane 120 mg of a pale yellow solid was obtained which was separated by preparative TLC at –20 °C with seven plates (Si 60: 20 × 20 cm, 200 μm, Merck) and pentane/hexane (80 : 20 v,v) as eluent.

The band at $R_f = 0.30$ was scraped off and eluted with dichloromethane. After evaporation 40 mg of pure compound were obtained. ^{1}H-spectrum, as well as the MS-spectrum were in agreement with literature data for α-(*E*,*E*)-farnesene (Murray, 1969).

Isolation of β-(E)-farnesene

350 mg of essential oil of *Chamomilla recutita* were separated by VLC on silica gel with pentane as the eluent. Final fractionation was obtained by HPLC with a silver nitrate impregnated cation-exchange column (Nucleosil 5 SA, Macherey and Nagel, 5 μm, 250 × 4 mm) and 1% acetonitrile in ethylether (van Beek and Subrtova, 1995). 45 mg of β-(*E*)-farnesene was obtained. ^{1}H- and ^{13}C-NMR spectra were in agreement with literature data for β-(*E*)-farnesene (Mendes and Silveira, 1994).

Gas chromatography

The gas chromatograph (HP G1800A, GCD System, Hewlett Packard, Palo Alto, USA) was

equipped with a S/SL injector (1:25, 250 °C) and an EI-MS detector (280 °C, 70 eV). Separation was achieved using a HP-5 fused silica column (15 m × 0.25 mm) using He as carrier gas (1 ml/min) and the following temperature programme: splitless, 40 °C isotherm for 5 min, then 40–200 °C at 10 °C/min, 200–300 °C at 30 °C/min.

Solid phase microextraction (SPME)

2.00 g (wet weight) of mistletoe flowers were transferred to 5 ml vials and sealed with a septum. The sample was kept for two hours at 30 °C. Then the fiber (100 μm) loaded with polydimethylsiloxane was introduced to the head space. After 30 minutes of exposure the fiber was retracted and injected into the gas chromatograph.

NMR spectroscopy

Spectra were recorded with a Bruker (Karlsruhe) AM 400 NMR-spectrometer at 400 MHz (^{1}H-NMR) and 100 MHz (^{13}C-NMR).

Quantitative analysis

1.00 g of flowers were crushed in liquid nitrogen in a mortar and transferred with ethylacetate to a 25 ml measuring flask. This was kept for one hour at room temperature with shaking every 5 min. 3 ml of the supernatent were injected to the gas chromatograph. Isolated α-[*E,E*]-and β-[*E*]-farnesene were used as external standards.

For SPME the relative intensity of the peak area was taken from the chromatograms.

Results and Discussion

Three kg of male mistletoe flowers grown on apple trees were extracted with dichloromethane. A combination of vacuum liquid chromatography and preparative thin layer chromatography at low temperature (–20 °C) led to the isolation of 40 mg of α-(*E,E*)-farnesene. α-(*E,E*)-Farnesene was the main compound of the volatile fraction of all mistletoe flowers tested by solid phase microextraction (SPME) and GC.

Whereas α-(*E,E*)-farnesene accounts for more than 90% of the volatiles for flowers from mistletoes grown on apple trees, it was observed that flowers from mistletoe grown on pine tree contained a few other less prominent volatiles components. This confirms the sensory impression that the flowers of the different host trees have a different odour. The MS of the GC peak at RT = 13.54 min suggested that it might be β-(*E*)-farnesene. Therefore we isolated the latter compound from a chemical variety of *Chamomilla recutita* rich in β-(*E*)-farnesene. The ^{13}C-NMR validated our hypothesis.

A GC peak at RT = 7.96 min could be attributed to linalool according to its MS spectrum and comparison with an authentic sample (Roth, Karlsruhe).GC peaks at RT = 6.97 min and 7.46 min showed mass spectra that were in agreement with that for cis- and trans-oxide of linalool respectively (according to Wiley data bank).

The intensity of the peaks in the gas chromatogram from the SPME experiments only reflect the relative amounts of the respective substances. We examined the absolute amount by extraction with ethylacetate and calibration with external standards of α-(*E,E*)-farnesene and β-(*E*)-farnesene. As can be seen (Table I) the content of α-(*E,E*)-farnesene varies with the host tree. It is highest for mistletoes grown on *Salix* trees. Female flowers are smaller and thus the amount of stem tissue per flower is higher; they contain less volatiles than for mistletoe flowers from *Malus* and *Salix*. The amount of β-(*E*)-farnesene can only be quantified from flowers of the *Pinus* host. The chromatograms from the ethylacetate extract show many less volatile compounds. The GC peaks of the farnesenes are therefore less dominant than in the SPME derived chromatogram. When we com-

Table I. Farnesene content in flowers from mistletoes grown on different hosts determined from an ethylacetate extract.

		μg farnesene/g flowers (fresh wt)*	
Host tree	Sex	α-(*E,E*)-farnesene (RT = 14.19)	β-(*E*)-farnesene (RT = 13.54)
Malus domestica	Male	59	–
Malus domestica	Female	38	–
Salix alba	Male	113	Tr
Salix alba	Female	47	–
Abies alba	Male	57	Tr
Pinus sylvestris	Female	42	14

*) Calculated from 2 independent assays with 3 repetitions.
Tr, trace, not quantifiable, see Table II.
–, Not quantifiable.

pared the absolute amount determined from the ethylacetate extract with the relative amount from the SPME experiments we found a good correlation. On this basis we could also quantify the amount of β-(*E*)-farnesene for *Salix* and *Abies* (see Table II).

Table II. β-(*E*)-Farnesene content of mistletoe flowers calculated from SPME-experiments.

		μg farnesene/g flowers (wet wt)	
Host tree	Sex	α-(*E,E*)-farnesene	β-(*E*)-farnesene
Salix alba	male	113	6.1
Abies alba	female	57	4.5

Luther P. and Becker H. (1987), Die Mistel. Botanik, Lektine, medizinische Anwendung. Springer Publ., Berlin, Heidelberg, p. 19–22.

Mendes F. N. P. and Silveira E. R. (1994), Fatty acids, sesqui- and diterpenes from seeds of *Dipteryx lacunifer*. Phytochemistry **35,** 1499–1503.

Murray K. E. (1969), α-Farnesene: Isolation from the natural coating of apples. Aust. J. Chem. **22**, 197–204.

Van Beek T.A and Subrtova (1995), Factors involved in the high pressure liquid chromatographic separation of alkenes by means of argentation chromatography on ion exchangers: Overview of theory and new practical developments. Phytochem. Anal. **6**, 1–19.

Wallden B. (1961), Misteln vid dess Nordgräns. Svensk Bot. Tidskr. **55,** 427–549.