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Short Communication

1,6-Germacradien-5-ol Identified in the Larval Discharge of the Pine Sawfly *Neodiprion Sertifer*

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We report the identification of the monocyclic sesquiterpene alcohol 1,6-germacradien-5-ol [systematic name (*Chem. Abstr.*): (2*E*,7*E*)-1,7-dimethyl-4-(1-methylethyl)-2,7-cyclodecadien-1-ol] in the larval protective discharge and in pupal and adult body parts of the pine sawfly, *Neodiprion sertifer* Geoffr. (Hymenoptera, Diprionidae) and in the needles and phloem of the two host pine trees, *Pinus silvestris* and *P. contorta*. The use of sequestered tree resins for defence by *N. sertifer* larvae has been described by Eisner, Meinwald and co-workers.[1] The chemical composition of the viscous droplets orally discharged by larvae has earlier been studied through the application of extraction and analytical techniques such as gas liquid chromatography (GLC), combined gas chromatography–mass spectrometry (GLC–MS) and liquid chromatography (HPLC). Resin acids, carbohydrates, monoterpenes (α- and β-pinene), diterpenes and phenolic compounds have been identified in the larval food (pine needles).[2–4] The amount of discharged liquid, which consists mainly of resin acids, was found to depend on the concentrations of resin acids in pine needles on which the larvae had been feeding.[5]

In a previous study we analysed the odoriferous secretion emitted from ventral glands of larch sawflies, *Pristiphora ericsonii* and *P. wesmaeli*.[6] The volatile compounds from these species are released in connection with characteristic defensive behaviour called 'snap bending'. *N. sertifer* larvae display similar group (communal) behaviour, but no associated odour has been noted, although they also possess ventral glands.

Materials and methods

Biological material. Droplets of larval regurgitate were collected from sawfly larvae feeding of *Pinus contorta* in a plantation near Österbymo, province of Östergötland, in the south of Sweden. The regurgitate was collected in 10 μl microcaps and immediately dissolved in redistilled pentane. At the same time, pine needles were collected and stored in liquid nitrogen pending chemical analysis. Pine needles and phloem samples were also gathered from another host plant, *P. silvestris*, at Landvetter near Göteborg on the Swedish west coast.

Chemical analysis. About 2 ml of the solution of regurgitate were analysed by GLC (Hewlett-Packard 5830) using a fused silica column 45 m × 0.32 mm ID, coated with OV-351 (d_f 0.5 μm). Temperature program: isothermal 50 °C for 5 min, and then programmed to 210 °C at 5 °C min^{-1}, and isothermal for 20 min; carrier gas: nitrogen at 18 cm s^{-1}. Identification of constituent compounds was performed by combined GLC–MS (Hewlett-Packard 5890 Finnigan TSQ 700 quadrupole mass spectrometer) furnished with an equivalent fused silica column coated with DB-wax (30 m × 0.25 mm). Temperature program: isothermal 50 °C for 2 min, then programmed to 200 °C at 8 °C min^{-1}, and isothermal for 15 min; carrier gas: helium at 25 cm s^{-1}. Volatile compounds in pine needles were isolated by cutting the needles into small pieces and extracting them in 1 ml pentane in an ultrasonic bath for 30 min, followed by further extraction at room temperature for 24 h; the resulting pentane extract was concentrated at room temperature to 0.5 ml in a tapered glass vial. Separation was performed by liquid chromatography on heat-activated (100 °C) silica, Merck 60 (0.063–0.200 mm, 70–230 mesh ASTM), with gradient elution by adding ethyl acetate to pentane from 1 to 10%, to a volume of 1.5 ml in 10 fractions.[7] Fraction 6 was analysed, without further evaporation, by GLC and GLC–MS. Phloem samples of about five cm^2 were prepared following the same procedure as for the pine needles.

Results and discussion

We initially found 1,6-germacradien-5-ol in *N. sertifer* when we compared the occurrence of pheromone-related compounds from different body parts of differently aged pupal and adult female *N. sertifer*. This alcohol, which

was isolated in liquid chromatography fraction number 6,[7] was found to occur in all pupal stages of larvae that had fed on *P. silvestris* (gas chromatogram in Fig. 1A). The amount in each individual increased with each pupal stage. Young pupae contained approximately 10 ng of germacradienol, medium-aged pupae about 200 ng, and old pupae maximal amounts averaging 500 ng; adult females contained only about one tenth of the amount in old pupae.

The chemical identification was first made tentatively by comparing the mass spectra of the natural compound with a reference spectrum of 1,6-germacradien-5-ol, courtesy of Dr. Daniel Joulain, Robertet SA, Grasse, France.[8] The compound was later provided as a reference sample from Dr. Roman Kaiser, Givaudan-Roure SA, Dübendorf, Switzerland,[9] which enabled us to identify the compound with certainty by comparing the mass spectra and gas chromatographic retention values with those of the reference sample. It also allowed the positive differention of germacradienol from the related tricyclic sesquiterpene 1-*endo*-bourbonanol, which produces a mass spectrum very similar to that of germacradienol, but with a distinctly different gas chromatographic retention value. Gas chromatographic retention values for the natural compound in needle extract was 31.94 min, and for germacradienol and bourbonanol in the reference sample 31.97 and 29.79, respectively.

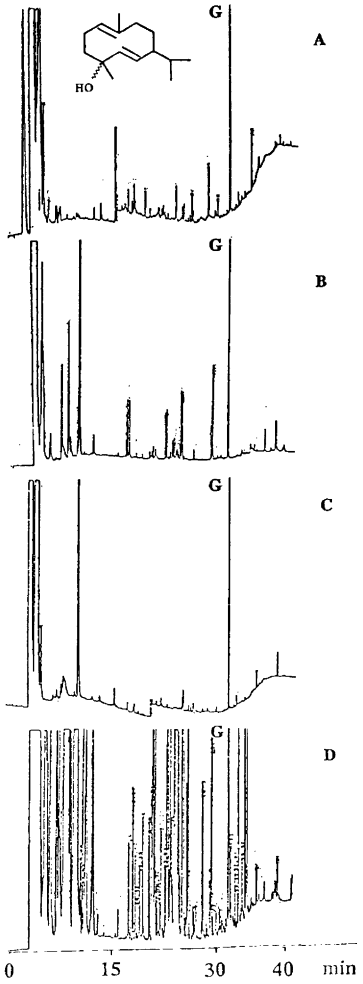

Fig. 1. Gas chromatograms of extracts containing 1,6-germacradien-5-ol (G). A, LC-fraction 6 of extracted *N. sertifer* pupae; B, extract of *N. sertifer* larval regurgitate; C, LC-fraction 6 of *P. contorta* needle extract; D, recombined LC-fractions 1–10 of the *P. contorta* needle extract.

The main compounds of the regurgitate volatiles from larvae feeding on *P. contorta*, shown in Fig. 1B, are 1,6-germacradien-5-ol (G) and monoterpene hydrocarbons, especially β-pinene, 3-carene and β-phellandrene. The GLC–MS analysis of LC-fraction number 6 of needle extracts from *P. contorta* also showed the occurrence of β-phellandrene ($t_R = 10.04$) and 1,6-germacradien-5-ol ($t_R = 31.97$), Fig. 1C. Differences in the composition of monoterpene hydrocarbons in the two pine species were noticeably large; the major monoterpenes in needles of *P. contorta* were β-pinene and β-phellandrene, while 3-carene and myrcene predominated in *P. silvestris*. The phloem extracts of both pine species contained large amounts of 1,6-germacradien-5-ol. In a GLC analysis of the recombined LC-fractions 1–10 of the *P. contorta* needle extract, shown in Fig. 1D, germacradienol makes up approximately 4% of the total volatile compounds. Retention ranges of groups of volatile terpenes are also evident in this chromatogram. The first region following the solvent peak contains monoterpene hydrocarbons (up to a retention time of 13.20 min); the next region (to 26.00 min) comprises oxygenated monoterpenes and sesquiterpene hydrocarbons; in the end region (to 39.00 min) sesquiterpene alcohols and diterpene hydrocarbons are present. A comparison between Figs. 1B and 1D indicates that the germacradienol can be selectively sequestered by the larvae. It may have an antimicrobial function.

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