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Advances in the identification and agrochemical importance of sesquiterpenoids from *Bulnesia sarmientoi* essential oil

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ABSTRACT

Bulnesia sarmientoi Lorentz ex Griseb volatile oil was characterized by GC × GC-TOF-MS analysis. Major components were guaiol and bulnesol, followed by hanamyol. The enhanced sensitivity and superior resolution of GC × GC resulted in the identification of thus-far unreported oil constituents as β-guaiene, guaioxide, elemol, germacrene-B, eudesm-5-en-11-ol, γ-eudesmol, α-eudesmol and (−)-hanamyol. The insecticidal effect of *B. sarmientoi* oil and its main constituents (guaiol, bulnesol and hanamyol) on *Spodoptera littoralis*, *Rhopalosiphum padi* and *Myzus persicae* was studied. Guaiol affected the aphids in a dose-response fashion, showing low efficiency, while bulnesol and hanamyol were inactive. Both the oil and its constituents were also assayed for antifungal action against *Fusarium* spp. and phytotoxicity to *Lactuca sativa*. Among the pure compounds tested, bulnesol had a low-moderate effect on *Fusarium moniliforme* while hanamyol had a strong effect on *Fusarium solani*. Neither the oil nor the tested compounds affected *L. sativa* germination or radicle length, indicating that *B. sarmientoi* is not phytotoxic.

1. Introduction

In the last years the fragrance industry has taken the approach of 'primary prevention' (i.e. restricting the use of the most widely acknowledged allergenic fragrance materials) (Bridges, 2002; Cadby et al., 2002). In addition, the biological activity of essential oils from medicinal plants – particularly their antibacterial, antifungal, and insecticidal properties – has been the subject of much research (Lemos et al., 1990; Gershenzon and Dudareva, 2007; Dudai et al., 1999; Isman, 2000). Major ingredients mediating the antibacterial and antifungal activity of essential oils have been isolated and identified (Lahlou, 2004).

A wealth of experience has been gathered on issues such as provenance and quality, safety, authenticity and on problems of isolation, processing and shelf life. On the basis of this fundament of knowledge, we should start to deal with those products whose composition resulted to be more complex when they are submitted to more sophisticated analytical approaches. This is clearly the situation with *Bulnesia sarmientoi* (palo santo) essential oil which composition, even known as complex, has not been well resolved until present through the analysis of the entire mixture.

B. sarmientoi Lorentz ex Griseb is a plant endemic to the region of Chaco in Argentina and Paraguay. It is commonly known as palo santo or as Paraguay lignum vitae – in view of its similarity to the lignum vitae trees of the genus Guaiacum (Mabberley, 1997). Guaiac essential oil is obtained by steam distillation of a mixture of *B. sarmientoi* wood and sawdust. The oil – a yellow to greenish semi-solid substance that melts at ca. 40–50 °C – has a soft rose-like odour, and has thus been used as an adulterant for rose oil (Guenther, 1944). Some costly derivatives of *B. sarmientoi* constituents – e.g. guaiol acetate – are used as fixatives in the manufacture of perfumes. Guaiac essential oil is typically a monoterpeneless oil containing high levels of two sesquiterpene alcohols: guaiol (**1**) and bulnesol (**2**) (Dolejs et al., 1961; Bates and Slagel, 1962; Wenninger et al., 1967; Prudent et al., 1991).

Using GC/MS, Prudent et al. (1991) reported minor components of the oil, such as guaiane or eudesmane sesquiterpenic alcohol isomers. However, using 1D-GC, the co-elution of compounds did

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not allow the characterization of a more complex composition, as suggested by newer studies based on different GC–MS methods. Nonetheless, a single analysis does not provide adequate resolution for the identification of several individual constituents (Marongiu et al., 2007).

The introduction of comprehensive two-dimensional gas chromatography (GC × GC) led to a substantial improvement in peak capacity (Dallüge et al., 2003; Adahchour et al., 2006; Adahchour et al., 2003; Cordero et al., 2007). The combined result of an increased separation capacity of GC × GC and the identification capability of TOF-MS enable the identification of unknown compounds, hence, the characterization of a number of substances whose composition may not be resolved by conventional procedures.

As a part of a study aiming at the incorporation of plant essential oils for use as low-risk agrochemicals, the effects of *B. sarmientoi* oil and its main constituents on various insect crop pests (*Spodoptera littoralis*, *Rhopalosiphum padi* and *Myzus persicae*) were studied, along with their antifungal action against certain plant pathogens (Fusarium spp.) and phytotoxicity to *Lactuca sativa*. 1D-GC–MS, comprehensive GC × GC-TOF-MS and some auxiliary methods were used to characterize commercial *B. sarmientoi*.

2. Materials and methods

2.1. Plant material

Authentic essential oil samples of palo santo were prepared by steam distillation by the *Sociedad Cooperativa Colonizadora Chortitzer Komitee Ltda.* (Loma Plata, Paraguay). All the oil samples were dissolved in ethyl acetate at a final concentration of 9.2 mg/ml. This solution was then diluted to 92 ng/μl and a mixture of *n*-alkanes (C_8–C_{20}) were added at a concentration of 80 pg/μl. For single-level-calibration an *n*-alkane standard solution of C_8–C_{20} was prepared at a concentration of 80 ng/μl. One microlitre from both solutions was injected splitless into the Pegasus 4D GC × GC-TOF-MS system.

2.2. Oil fractionation

Liquid chromatography was used for the isolation and fractionation of the target compounds. 6.35 g of the essential oil was diluted in ethyl acetate (Riedel-de-Häen, Germany) and subjected to open-column chromatography on silica flash (1:60 w/w) using a Büchi Sepacore Flash device (Büchi Labortechnik AG, Postfach, Schweizand) and was then eluted with hexane and different proportions of hexane-EtOAc. This led to the separation of fourteen fractions accounting for 94,9% (w/w) of the oil sample. These fractions were analysed by IR, ^{1}H and ^{13}C NMR. Hanamyol was obtained by crystallization from the fraction in which it was purer (fraction 14).

2.3. IR, ^{1}H and ^{13}C NMR analysis

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, as solvent unless otherwise stated, at 300/500 MHz or 75/125 MHz, respectively, with on Varian Mercury 300 or Bruker DMX-500 instruments. IR spectra were recorded on a MATTSON Genesis II FTIR. Optical rotation values were measured on a Perkin Elmer 341 instrument, while for X-ray structure analysis cell constants and intensities were measured on a Bruker Apex-II CCD automated diffractometer.

2.4. GC–MS analysis

For identification purposes, 1D-GC–MS analyses were conducted using a Shimadzu QP 5050 apparatus equipped with reference libraries (Adams, 2007; McLafferty and Stauffer, 1991) using an SE-52 (Mega) crosslinked fused-silica capillary column (25 m, 0.25 mm i.d.) coated with 5% phenyl-polymethylsiloxane (0.25 μm phase thickness) following the experimental conditions previously reported by Frizzo et al. (2008).

For GC × GC-TOF-MS analysis, the system was a LECO Pegasus 4D (LECO Corporation, St. Joseph, MI), consisting of an HP 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), dual stage cryogenic jet modulator, secondary oven, LECO TOF-MS detector equipped and a CTC autosampler (CombiPAL CTC Analytics AG, Switzerland). The first dimension column was an Equity-1 (Supelco, USA) crosslinked fused-silica capillary column (60 m × 0.25 mm i.d.), coated with 100% polydimethylsiloxane (0.25 μm phase thickness). The second dimension column was a BPX-50 (SGE, Australia) crosslinked fused-silica capillary column (2.5 m, 0.10 mm i.d.), coated with 50% phenyl, 50% dimethylpolysiloxane (0.1 μm phase thickness). The GC was operated under the conditions previously described (Dellacassa et al., 2008).

Analysed samples were processed by means of an automated peak finding and deconvolution function. Within automated data processing, the ChromaTOF software automatically detects peaks at each acquired single mass above a certain signal-to-noise level. After that, spectral deconvolution and automated combination of modulated peaks are performed. Mass spectra are compared to NIST library and the peaks are identified.

In our work, we used automated data processing as the first step, followed by manual work with the data such as the plotting of characteristic masses to find the peaks of interest and also manual identification of the spectra missing in the library. Retention indices (1D chromatogram) were calculated using an homologous series of *n*-alkanes added to the palo santo essential oil. Calculated retention indices were compared to the literature values (McLafferty and Stauffer, 1991; Frizzo et al., 2008; Jennings and Shibamoto, 1980.). To automatically semi-quantify the compounds of interest, a calibration curve was built with *n*-hexadecane ($y = 790.222x$; $r^2 = 1$).

2.5. Insect bioassays

S. littoralis (Boisduval) (Lepidoptera: Noctuidae), *M. persicae* (Sulzer) and *R. padi* (Hom.), (Hemiptera: Aphididae) colonies were reared on artificial diet, bell pepper (*Capsicum annuum*) and barley (*Hordeum vulgare*) plants, respectively, and maintained at 22 ± 1 °C, >70% relative humidity with a photoperiod of 16–8 h (L:D) in a growth chamber.

2.6. Choice feeding assay

The experiments were conducted with *M. persicae* and *R. padi* (apterous) adults and sixth-instar *S. littoralis* larvae. Feeding or settling inhibition (%FI or %SI) was calculated as described by Reina et al. (1997). The active compounds were tested in a dose-response experiment to calculate their relative potency (EC_{50} values, the effective dose to give 50% inhibition), which was determined from linear regression analysis (% inhibition on log dose). The experiments were carried out in triplicate.

2.7. Oral cannulation

This experiment was performed with pre-weighed newly molted *S. littoralis* L6-larvae. Each experiment consisted of 20 lar-