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Towards a complete characterisation of guaiacwood oil

Loïc Tissandié [a], Stéphane Viciana [a], Hugues Brevard [b], Uwe J. Meierhenrich [a], Jean-Jacques Filippi [a, *]

[a] Institut de Chimie de Nice, Université Côte d'Azur, UMR 7272 CNRS, Parc Valrose, 06108 Nice Cedex 2, France
[b] Robertet S.A., 37 Avenue Sidi Brahim, 06130 Grasse, France

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ABSTRACT

Guaiacwood oil is a common perfume ingredient used in modern compositions for its suave woody-rosy scent. This essential oil is a byproduct of the timber industry obtained by hydrodistillation of the heartwood of *Bulnesia sarmientoi*, a tree native from Latin America. Despite being widely used in perfumery, guaiacwood oil has been poorly described in the past. This study aims at giving an in-depth characterisation of its chemical composition as well as disclosing the odorant compounds responsible for its characteristic fragrance. Our methodology was based on a combination of fractionation and analytical techniques, including comprehensive two-dimensional gas chromatography coupled to mass spectrometry and preparative capillary-gas chromatography. The entire analytical work led to the isolation of 20 constituents among which 14 have never been reported so far in natural extracts. Each isolated compound was fully characterised by spectroscopic methods. Finally, the accurate knowledge of the chemical composition permitted the identification of the odour-active constituents by gas chromatography-olfactometry.

1. Introduction

Bulnesia sarmientoi (Zygophyllaceae) Lorentz ex Griseb, also known as "*Palo Santo*" in Latin America, is an endemic tree from the Gran Chaco region that furnishes a fragrant wood, long used by the local population as a multipurpose folk medicine. Owing to its particularly high density and its high content in resin, guaiacwood also serves as a proper material for cattle fences, but also in parquetry and various handicrafts (Waller et al., 2012). The essential oil known as guaiacwood oil (GWO) is obtained as a byproduct of its forest exploitation by distillation of sawdust and wood leftovers. This very viscous essential oil is highly prized by the cosmetic and perfume industry for its excellent fixative properties and its delicate woody-rosy odour (Arctander, 1960; Surburg and Panten, 2006). However, despite being a very common perfume ingredient, its chemical composition is partially known and the characterisation of its odour-active constituents is missing. As often observed for woody fragrant oils (Baldovini et al., 2011; Belhassen et al., 2015; Naef, 2011; van Beek and Joulain, 2018), GWO is almost exclusively composed of sesquiterpene derivatives, mostly distributed within the guaiane and eudesmane families. In contrast with oils of vetiver, patchouli, or agarwood, GWO is largely dominated by tertiary alcohols where guaiol (**74**) and bulnesol (**111**) generally represent more than two thirds of the oil (Prudent et al., 1991; Rodilla et al., 2011). In addition to these two alcohols, β-elemol (**61**), 10-epi-γ-eudesmol (**87**), γ-eudesmol (**89**), α-eudesmol (**107**), and β-eudesmol (**108**) were reported as major constituents. All of these compounds are easily identified by gas chromatography-mass spectrometry. However, because of a high extent of coelutions, the identification of minor compounds in GWO remains a complex task. Owing to its resolutive power and high sensibility, comprehensive two-dimensional gas chromatography (GC × GC) is thus particularly adapted for the analysis of complex mixtures such as essential oils (Filippi et al., 2013). Thus, the analysis of GWO by GC × GC permitted us to obtain a detailed information on its chemical composition. In addition, GC × GC provided a distribution of the analytes in distinct chromatographic regions of the 2D-plot. Two particular areas—hydrocarbons and oxides (RI VF-5ms 1400–1600) and tertiary alcohols (RI VF-5ms 1650–1700)—were determined to contain several unknown compounds (Fig. 1). In a previous report, a particular attention was paid

* Corresponding author.
E-mail address: jfilippi@unice.fr (J.-J. Filippi).

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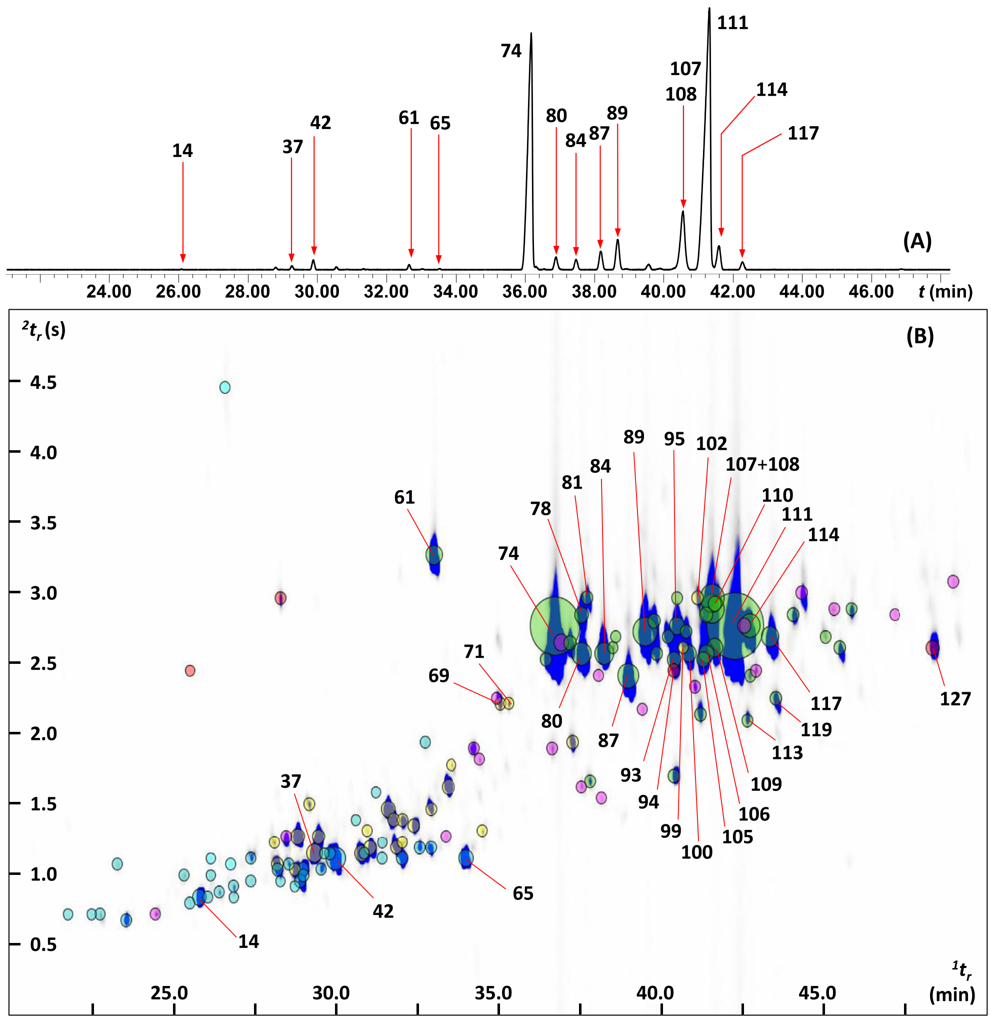

Fig. 1. (A) GC–MS analysis of GWO pointing the major sesquiterpenes and (B) GC × GC–MS analysis of the same sample, additionally displaying the isolated compounds. Sesquiterpene hydrocarbons appear as pale blue blobs, sesquiterpene oxides appear as yellow blobs, sesquiterpene alcohols appear as green blobs, ketones are displayed in pale red, whereas unknown compounds appear as pink blobs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

to the identification of minor sesquiterpene oxides, resulting in the characterisation of a full series of 5,11- and 10,11-epoxyguaiane derivatives and agarofurans (Tissandié et al., 2017). In the present study, we report an in-depth analysis of GWO, aiming at giving the most complete characterisation of its chemical composition, and particularly targeting its odoriferous constituents. For this purpose, several sesquiterpenic constituents, mostly tertiary alcohols present at trace level, were isolated by preparative capillary-gas chromatography (pc-GC) and their structures were fully elucidated by spectroscopic methods. The detailed qualitative and quantitative analysis of the oil was finally carried out by means of comprehensive two-dimensional gas chromatography (GC × GC–FID/MS) while the determination of its odour-active constituents was achieved by gas chromatography-olfactometry techniques (GC–O and GC-MS–O).

2. Results and discussion

2.1. *Isolation of unknown alcohols*

In order to carry out the isolation of the unknown tertiary alcohols, GWO was first fractionated on silica gel into eight fractions (F1–F8) using a solvent gradient of petroleum ether and diethyl ether. The alcohol fractions F3–F6 were repeatedly chromatographed either on standard or $AgNO_3$-impregnated silica gel (see Experimental Section). The obtained subfractions were then used in preparative capillary-gas chromatography (pc-GC) to isolate unknown compounds, whenever possible. Compounds **80** and **110** were directly isolated from F4 and F6 fractions, respectively, with sufficient purity for NMR analysis. In total, 20 compounds were isolated and fully characterised by MS and NMR data analysis (Supporting Information). Among the isolated constituents, several hitherto unknown tertiary alcohols were identified. A comparative description of their ^{13}C NMR data is given in Tables 1 and 2.

The isolated compounds belonged mainly to the family of guaianes. Among them, compound **80** provided a mass spectrum very close to that of guaiol (**74**). In addition, its NMR data indicated that **80** shared the same basic structure with **74**, but its NOESY clearly indicated that methyl-10 was oriented in α-position. Hence, **80** was identified as 10-epi-guaiol. The co-occurrence of the two epimers **74** and **80** in GWO is not surprising and might well originate from the isomerisation of bulnesol (**111**) during extraction of the oil. Previous studies on GWO already suggested the presence of an epimer of guaiol (**74**) among the constituents of the essential oil, nonetheless this compound has never been properly isolated (Prudent et al., 1991; Rodilla et al., 2011). 10-epi-Guaiol was