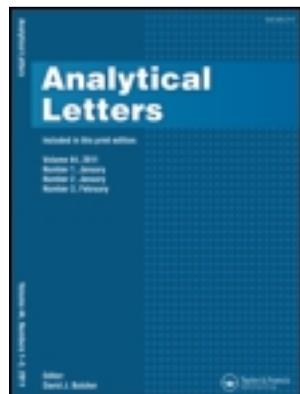


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Analytical Letters

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/lanl20>

The Famous Amazonian Rosewood Essential Oil: Characterization and Adulteration Monitoring by Electrospray Ionization Mass Spectrometry Fingerprinting

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Available online: 06 Oct 2011

To cite this article: Rita C. Z. Souza, Marina Marques Eiras, Elaine C. Cabral, Lauro E. S. Barata, Marcos N. Eberlin & Rodrigo R. Catharino (2011): The Famous Amazonian Rosewood Essential Oil: Characterization and Adulteration Monitoring by Electrospray Ionization Mass Spectrometry Fingerprinting, *Analytical Letters*, 44:15, 2417-2422

To link to this article: <http://dx.doi.org/10.1080/00032719.2011.551852>

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Mass Spectrometry

THE FAMOUS AMAZONIAN ROSEWOOD ESSENTIAL OIL: CHARACTERIZATION AND ADULTERATION MONITORING BY ELECTROSPRAY IONIZATION MASS SPECTROMETRY FINGERPRINTING

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Direct infusion of samples via electrospray ionization mass spectrometry (ESI-MS) is shown to characterize unequivocally genuine samples of Amazonian Aniba rosaeodora Ducke (Rosewood) essential oils obtained either from the wood or leaves. The ESI-MS also distinguishes the essential oils from synthetic linalool; hence, adulteration by the synthetic oil is also clearly detected. The analysis requires no pretreatment or preseparation, and the most polar components of the essential oil are extracted with an acidified 1:1 methanol water solution. This simple extract is then analyzed by direct infusion ESI-MS in the positive ion mode, which provides characteristic fingerprintings of the sample composition. The ESI-MS fingerprinting can be used therefore as a simple and fast (few minutes) method for authenticity and quality control of this famous Amazonian essential oil.

Keywords: Biomarkers; Electrospray; Essential oil; Fingerprintings; Quality control

INTRODUCTION

Odoriferous plants are a vital part of Amazonians' daily life and of their economic activities (Zellner et al. 2006). Among these unique plants, Rosewood (*Aniba rosaeodora* Ducke) still provides the most commercially important essential oil from the Amazon. Present in Chanel perfumery since the 1930s, the Rosewood essential oil is

Received 23 March 2010; accepted 17 November 2010.

This work was supported by the São Paulo State Research Foundation (FAPESP) and the Brazilian National Research Council (CNPq).

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now the best representative of predatory exploitation and, as a result, Rosewood trees are currently in danger of extinction (Zellner et al. 2006). More than 2 million trees have already been chopped down and its exploration has contributed to depletion of the Amazon forest. For the production of approximately 40 tons of oil, nowadays approximately 4,000 trees are taken down every year. Produced through steam distillation (1% yield), Rosewood essential oil costs about US\$80/kg (FOB, Manaus) and, despite the predation negative aspect, the oil is still used by the perfumery houses.

An environmentally friend new source of Rosewood oil would, therefore, be very welcomed. Fortunately, we found that Rosewood's leaves provide an equivalent essential oil by steam distillation. Comparative analyses via chiral GC and sensorial panels by perfumers revealed that the Rosewood leaf oil could replace the wood oil in fine perfumery. Sensorial analysis showed that the crude Rosewood leaf oil presents an aroma classified as sweet, floral, and rose/petit grain-like. Rectification of Rosewood leaf oil by re-distillation provides a very fragrant linalool with a greater woody note (Zellner et al. 2006). The quantitative differences in composition between wood and leaf oils were recently determined via GC-MS and enantioselective GC-olfactometry (Zellner et al. 2006), estimating adequately the olfactory activity of each linalool antipode.

Rosewood wood oil has experienced a long downward trend since the 1960s, when it was replaced by synthetic linalool in the lower grade perfumery market. In the 1970s, Rosewood's market share was further eroded by Chinese Ho (*Cinnamomum camphora*) wood and leaf oils, which replaced Rosewood oils in mid-range perfumes, cosmetics, and household products. Today, Rosewood essential oil and its derivatives are only used as main scent in a few top perfumes, and as a component of *bouquets* in a wider range of scents. Synthetic linalool is widely available at a low price of US\$6–8 kg⁻¹, and Ho oil for \$10–12 kg⁻¹, thus easily undercutting Rosewood oil in most fragrance applications. A common practice is therefore to blend the fine genuine Rosewood oil with inexpensive synthetic linalool.

We have applied direct infusion electrospray ionization mass spectrometry (ESI-MS) for the fingerprinting characterization of essential oils (Moller, Catharino, and Eberlin 2007). This technique has been proved to be versatile and fast and is applied with no pre-separation and with little or no sample preparation. It establishes a chemical fingerprinting for the composition of major polar compounds. ESI-MS fingerprinting has been used for a variety of samples such as vegetable oil (Catharino et al. 2005; Riccio et al. 2011; Saraiva et al. 2009; Simas et al. 2010; Wu, Rodgers and Marshall 2004), fruits (Roesler et al. 2007), beer (Araujo et al. 2005), wine (Biasoto et al. 2010; Catharino et al. 2006; Cooper and Marshall 2001), whisky (Moller, Catharino, and Eberlin 2005), soybeans (Santos et al. 2006), yerba mate × green tea constituents (Bastos et al. 2007), and biodiesel (Catharino et al. 2007).

Herein, we investigate the ability of ESI-MS fingerprinting to characterize Amazonian Rosewood essential oil either from crude wood or leaf and to detect adulteration with the addition of synthetic linalool.

MATERIALS AND METHODS

Chemicals

Methanol of analytical HPLC grade (Tedia) and formic acid (Merck) were used.

Materials

Fifteen samples of essential oil from crude wood oil and crude leaf oil of *Aniba rosaeodora* Ducke, as well as synthetic linalool. The crude essential oil of rosewood wood and leaves, as well as their linalool-rich distilled fractions, were supplied by Prof. Dr. Lauro Barata (Universidade Estadual de Campinas, São Paulo, Brazil) and the following standard compound was purchased from Sigma-Aldrich (Bellefonte, PA). For the detection of adulteration of crude wood oil with synthetic linalool, a mixture of these samples was prepared. All the samples were stored at 4°C.

Electrospray Ionization Mass Spectrometry

A Q-TOF mass spectrometer (Waters Micromass, Manchester, UK) was used for fingerprinting ESI-MS analysis. The general conditions were: Source temperature of 100°C, capillary voltage of 3.0 kV and cone voltage of 20 V. The oil sample (250.0 µL) was homogenized in a flask with equal parts of water and methanol, completing the final volume of 1.0 mL. The phases were allowed to separate, and the top (hydroalcoholic) layer was removed. For ESI(+)-MS analysis, 10.0 µL of concentrated formic acid were added to the sample mixture presenting a total volume of 1000 µL (0.1% for the final concentration). The extract was then injected directly to the ESI source at a flow rate of 10 µL min⁻¹ using a syringe pump (Harvard Apparatus). Mass spectra were acquired and accumulated over 60 sec and over the 50 to 1000 *m/z* range.

Tandem Mass Spectrometry

Structural analysis was performed by ESI-MS/MS. The ion of an *m/z* of interest was selected and submitted to 15–55 eV collisions with argon in the collision quadrupole. The collision energy and gas pressure were optimized to produce extensive fragmentation of the ion under investigation.

Data handling. All data obtained from ESI-MS of the various essential oils were dealt with using MassLynx 3.5 (Waters, Manchester, UK). Mass spectral data was accumulated over approximately 20 sec and the relevant mass range was selected and enlarged, depending on the MS mode ranging from *m/z* 50 to 500 or 1000, respectively (a range that contained all ions of interest as determined by visual inspection).

Statistical Analysis of Data

Principal Component Analysis (PCA) was performed using the 2.60 version of Pirouette software (Infometrix, Woodinville, WA, USA). The mass spectra were expressed as the intensities of individual [M + H]⁺ ions (i.e., variables). All the ions were included.

RESULTS AND DISCUSSION

Figure 1 shows the ESI(+)-MS of the extracts of crude Rosewood oils (from both the wood and leaf) and that of synthetic linalool. The two crude essential oils display very similar ESI(+)-MS profiles, with more intense ions of *m/z* 137, 153, 203, 221,

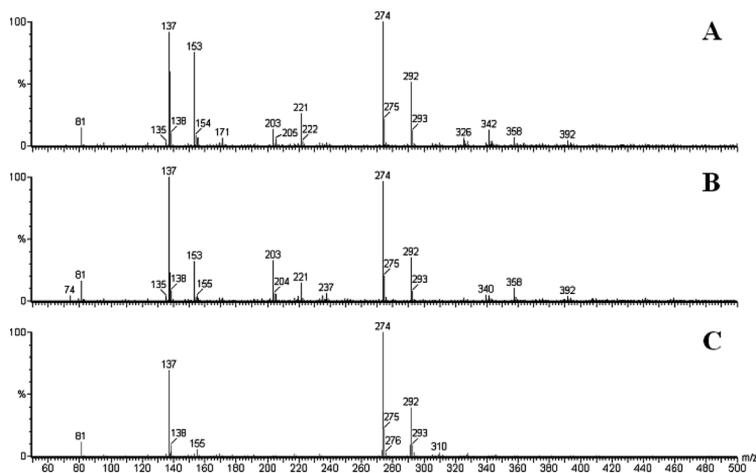


Figure 1. ESI(+)-MS of a) crude Rosewood wood oil, b) crude Rosewood leaf oil, and c) synthetic linalool.

274, 292, and 358 (Figures 1A and 1B). The ESI-MS from the synthetic linalool (Figure 1C) differs considerably from the two crude oils. The most important and peculiar difference is the absence of a number of marker ions of the essential oils, that is, those of m/z 153, 203, 221, and 358. In these spectra, the ion of m/z 137 arrives likely from ionization via protonation and water loss of linalool, which explains the very low intensity of protonated linalool of m/z 155. The ion of m/z 292 is attributed to the adduct formed from gas-phase addition of neutral linalool to the ion of m/z 137 whereas that of m/z 274 is likely formed by water loss from the ion of m/z 292. The ion of m/z 153, detected in the ESI-MS of both crude oils, is attributed to the protonated molecule of dehydrated linalool oxide whereas its precursor ion, that is, protonated linalool oxide of m/z 171 is minor, and can be observed mainly in the ESI-MS of the crude wood oil. The marker ions of m/z 221 and 203 of the two crude oils are likely the protonated molecules of spathulenol and dehydrated spathulenol, respectively.

Although not essential for fingerprinting, ESI(+)-MS/MS (not shown) of a few or each one of these marker ions can also be acquired (automatically) to provide bi-dimensional ESI-MS/MS fingerprinting selectivity for Rosewood oil analysis.

Although both crude oils display similar ESI-MS profiles, they can also be distinguished via some characteristic abundance ratios. For instance, the abundance ratio for the ions of m/z 137 and 153 is 1.2:1 for the wood oil and 3.1:1 for the leaf oil, whereas for synthetic linalool the ion of m/z 153 is not detected at all (Figure 1). For the ions of m/z 203 and 221, the ratios are 1:2 for the wood oil and 2.3:1 for the leaf oil, whereas for synthetic linalool the ions of m/z 203 and 221 are again not detected at all.

The ability of ESI-MS fingerprinting to detect Rosewood oil adulteration was also tested. When the genuine wood oil was spiked with just 10% of synthetic linalool (Figure 2), the ESI-MS changed noticeably. For instance, the abundance ratio for the ions of m/z 137 and 153 dropped about 5 times, from 1.2:1 to 1:2.2.

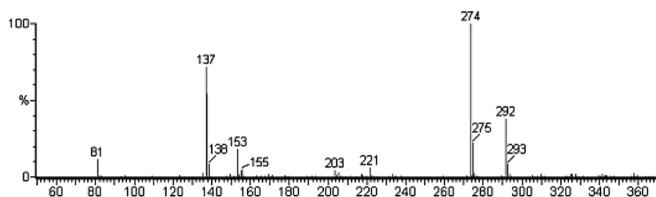


Figure 2. ESI(+)-MS of the crude Rosewood wood oil spiked with 10% synthetic linalool. Compare with the ESI-MS of the genuine oil in Figure 1A.

PCA Analysis

As discussed already, visual inspection of ESI-MS data allows one to unambiguously recognize wood, leaf, and synthetic linalool oils and their mixtures. But, as Figure 3 clearly illustrates, this ability can be further improved as well as automated by PCA analysis, which places samples in three very well-defined groups. Note that samples of the genuine wood oil adulterated with just 10% of synthetic linalool display ESI-MS fingerprintings very similar to those of pure synthetic linalool, and they fall therefore in the PCA plot in the synthetic linalool group. However, visual inspection of the ESI-MS can still detect the marker ions from the essential oil.

The use of ESI-MS fingerprinting provides a fast, simple, and reliable method able to provide characteristic snapshots of chemical composition that distinguish between genuine samples of the famous Amazonian Rosewood essential oil obtained either from the wood or leaves as well as their adulteration with synthetic linalool.

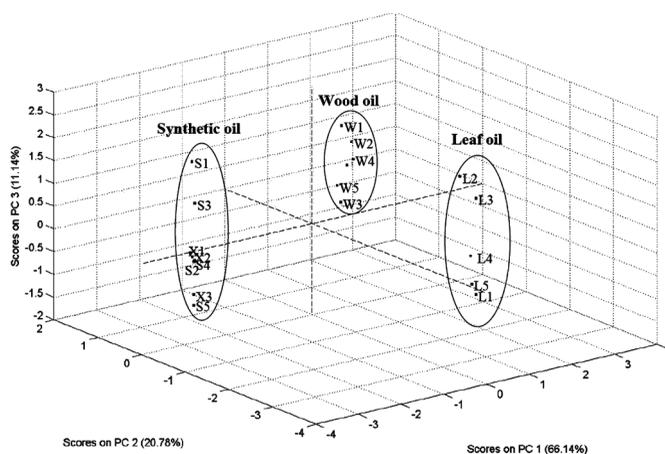


Figure 3. PCA analysis of the ESI-MS data for samples of Rosewood wood and leaf oils and synthetic linalool: wood (W1–W5), leaf (L1–L5), synthetic (S1–S5), and mixtures of wood and synthetic oils (X1–X3).

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