COMPOSITION OF THE VOLATILE COMPOUNDS FROM *ANIBA CANELILLA* (H. B. K.) MEZ. EXTRACTED BY CO$_2$ IN THE SUPERCRITICAL STATE

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ABSTRACT

The volatile compounds obtained by SFE-CO$_2$ (supercritical fluid extraction utilizing CO$_2$) from the barks of *Aniba canelilla* (H. B. K.) Mez. (Lauraceae) were analyzed by HRGC-FID (high resolution gas chromatography - flame ionization detector) and HRGC-MS (high resolution gas chromatography - mass spectrometry). Phenylpropanoids and lower amounts of sesquiterpenoids, representing *ca.* 97% of the total oil, were identified. The main compound, 2-phenylnitroethane, corresponds to 71,12% of the total oil.

Keywords: *Aniba canelilla* (H.B.K.) Mez., Lauraceae, SFE-CO$_2$, HRGC-FID, HRGC-MS, phenylpropanoids, sesquiterpenoids

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INTRODUCTION

The volatile compounds from plants are a class of compounds related to aspects such as pharmacological or biological/ecological activities, and to the organoleptical properties from several plants utilized in food or cosmetics and perfumery industry (1). The need for analytical tools which allows a faster and accurate analysis of these compounds from smaller amounts of plant material, leaded to an increasing utilization of SFE (supercritical fluid extraction), usually coupled (*on line* or *off line*) with instrumental chromatographic techniques (2).

SFE is an extraction technique, whose applications are growing in the field of analytical chemistry of natural products, as it is shown by reviews and a special issue of *Phytochemical Analysis* (2) dedicated to SFE. Its importance in the fast analysis of essential oils from plants is already recognized, since that the extracts obtained with supercritical CO$_2$ are usually similar to those obtained by classical steam distillation (2,3). Despite the latter being a well known method and one of the cheaper procedures for industrial extraction of volatile compounds from plants, steam distillation offers some disadvantages in comparison to SFE at the analytical point of view, such as the longer extraction times required for exhaustive extraction, the relatively great amount of sample required for extraction and the possibility of degradation and/or isomerization of thermolabile compounds (4). SFE also offers the possibility of *on line* coupling with instrumental chromatographic techniques, such as GC-MS (gas chromatography - mass spectrometry), which is still the more powerful technique of identification of volatile and low molecular weight compounds.

In a previous work we have shown that analytical scale CO$_2$-supercritical fluid extraction (SFE-CO$_2$), using a home-made system, can be a fast and inexpensive alternative sample preparation method for extraction of volatile compounds from plants, with analytical purposes such as HRGC-MS (high resolution gas chromatography - mass spectrometry) analysis (3). The present work reports the composition of the volatile compounds of *Aniba canelilla* (H.B.K.) Mez. (Lauraceae), which were obtained by SFE-CO$_2$. This extract was analyzed by HRGC-FID (high resolution gas chromatography - flame ionization detector) and HRGC-MS (high resolution chromatography - mass spectrometry), respectively for quantitative analysis of the main components and their identification.

Several Brazilian members of the Lauraceae family have a

significant content of essential oils, some of them being utilized in perfumery or as spices in foods. The barks of *A. canelilla* have a cinnamon-like odor, due to the unusual compound, 2-phenylnitroethane (5), which showed to be the main compound (6). Other major compounds are eugenol and methyleugenol (6); however, the identities of other volatile compounds from *A. canelilla*, as well as their relative content, have never been reported in the literature.

EXPERIMENTAL

Plant material

Trunk barks of *Aniba canelilla* (H.B.K.) Mez. (Lauraceae) were collected at Ilha do Cardoso, south coast of São Paulo state, Brazil, by Dr. Mario Motidome, Instituto de Química, Universidade de São Paulo, Brazil, and classified by Prof. Klaus Kubitsky, Universität Hamburg, Germany, where a voucher sample is deposited.

Plant material was dried at 40°C, with forced ventilation during 3 days before being powdered and sieved. Only particles between 0.21 - 0.35 mm were utilized for the extractions.

Extraction of the volatile compounds (SFE-CO$_2$ extract)

The SFE-CO$_2$ extract was obtained in a home-made system built at the IQSC-USP, previously described (3): liquefied CO$_2$ (Liquid Carbonic) was utilized as the extraction fluid. Extractions were performed above the critical constants of CO$_2$ (Tc= 31.3°C, Pc= 72.9 atm), at 80 atm and 60°C for 40 min. The dimensions of extraction cell (stainless steel) were 20 cm length x 0.7 cm i. d. Samples of 3 g each were extracted. The extracts were trapped in analytical grade CHCl$_3$ (Merck, ca. 20 ml, at 25°C). After extraction, the CHCl$_3$ was evaporated at room temperature (yield: 0,10 g/kg *A. canelilla* powdered trunk bark).

Gas chromatography (HRGC-FID)

The SFE-CO$_2$ extract was directly analyzed, without any prior clean-up procedure. The extract was solubilized in analytical grade CHCl$_3$ for chromatographic analysis.

HRGC-FID analysis were performed on a HP 5890 GC with a HP-1 cross-linked methyl silicone column (12 m x 0.25 mm i. d., 0.3 mm film thickness), supplied by Hewlett-Packard, USA. Samples were injected

using the split mode (split ratio 1:30), with injector and detector both at 280°C. Column temperature was programmed from 60°C (2min), at 5°C/min to 280°C. Hydrogen was used as carrier gas at 40 cm/sec. Quantification of each compound was done by area normalization method.

Gas chromatography-Mass spectrometry (HRGC-MS)

The SFE-CO$_2$ extract was also directly analyzed, without any clean-up and after being solubilized in analytical grade CHCl$_3$.

HRGC-MS analysis were performed using a HP 5970 mass selective detector operating in the electron impact mode (70 eV), coupled to a HP 5890 GC. The column utilized was an HP-5 cross-linked phenyl (5%) methyl (95%) silicone (25 m x 0.25 mm i. d., 0.3 mm film thickness), supplied by Hewlett-Packard, USA. Samples were injected using the split mode (split ratio 1:30), with the injector temperature and GC-MS interface both maintained at 280°C. The column temperature was programmed from 60°C (2 min), at 5°C/min to 280°C. Hydrogen was used as carrier gas at 40 cm/sec. MS scan range was 40 to 400 a.m.u. Data were processed on a HP 7946/HP 9000-300 CPU.

Identification of the compounds

Identifications were made by direct comparison of retention times with those of standards and by analysis of MS fragmentation data (7); *tentative identifications* were done by searching at the NBS library (ca. 40,000 registers) and analysis of fragmentation patterns. Crude extract was also analyzed by NMR, and spectra were obtained on a Brücker AM-200 spectrometer, at 200 MHz, using CDCl$_3$ as solvent.

RESULTS AND DISCUSSION

A. canelilla barks yielded 0.10 g/kg of volatile compounds, corresponding to the essential oil. Fourteen compounds were identified by means of their retention times and mass spectra. Table 1 shows the percentage of each identified compounds in the oil, determined using HRGC-FID (Figure 1).

The previously reported 2-phenylnitroethane is the major component (71,12%) and had its identity confirmed by NMR spectral analysis of the total SFE-CO$_2$ extract. The predominance of phenylpropanoid compounds - including eugenol ·(3,97%) and

Table 1. Chemical composition of the volatile compounds from *Aniba canelilla* (H. B. K.) Mez, obtained by SFE-CO_2

Peak	Compound	Identification[b]	GC-FID area (%)
1	2-phenylnitroethane	MS, NMR	71. 12
2	eugenol	MS, R_t	3. 97
3	methyleugenol	MS, R_t ·	9. 26
4	curcumen	MS	0. 33
5	$C_{15}H_{24}$[a] ·	MS	0. 23
6	$C_{15}H_{24}$[a]	MS	0. 19
7	$C_{15}H_{24}$[a]	MS	0. 44
8	calamalen	MS	0. 24
9	cadinen	MS	0. 14
10	g-eudesmol	MS	1. 99
11	$C_{15}H_{26}O$[a]	MS	2. 01
12	bisabolol	MS, R_t	7. 02

[a] tentative identification
[b] MS: analysis of fragmentation pattern
  NMR: analysis of crude extract by ^{1}H NMR (200 MHz)
  R_t: comparison of retention times with those of authentic compounds
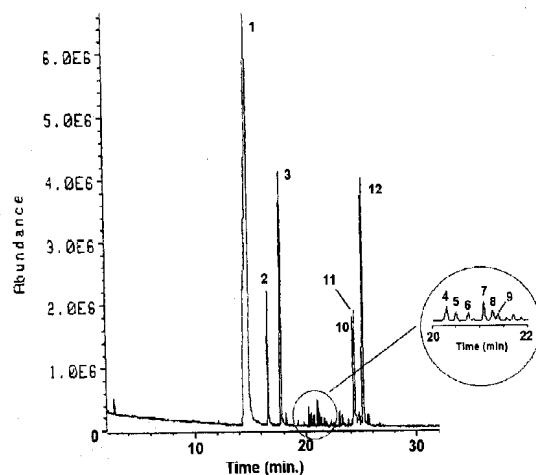
Figure 1. TIC-MS-HRGC chromatogram of the SFE-CO_2 extract from *Aniba canelilla* (H. B. K.) Mez. barks. For compound identification see Table 1. ·

methyleugenol (9,26%) - is the more important feature of the chemical composition profile of the *A. canelilla* oil. This could be expected since the genus *Aniba* is characterized by a C_6C_3 chemistry. In addition, small quantities of sesquiterpenes (hydrocarbons and oxygenated) were found. Despite the fact that some of those sesquiterpenoids were previously found in the essential oil of other Lauraceae species, these compounds are not abundant in the genus *Aniba* (8).

The data herein presented were obtained from *off line* SFE-HRGC-MS. This step is being studied in our laboratory as the preliminary stage for further *on line* SFE-HRGC-MS analysis, utilizing extraction and interface systems being developed and built in our laboratory, with applications including natural products (9). The development of *off line* or *on line* analytical methods for volatiles compounds from plants must also be considered at the point of view of reduction of costs (time and reagents consumption): for instance, in the case of *Aniba canelilla*, the data here shown indicate that SFE can be a tool for fast quality control of its essential oil: the predominance of 2-phenylnitroethane, followed by methyleugenol and eugenol is in agreement with the chemical profile from the essential oil (possibly obtained by steam distillation) (6). A 2-phenylnitroethane is undoubtedly a good marker for this specie (since that it is a very rare compound), it is possible to consider SFE-CO_2 as a valuable tool for a fast analysis of authenticity of *A. canelilla* samples.

RESUMO

Os constituintes químicos voláteis das cascas de *Aniba canelilla* (H.B.K.) Mez. (Lauraceae), obtidos por SFE-CO_2 (extração com fluido supercrítico utilizando CO_2), foram analisados por HRGC-FID (cromatografia gasosa de alta resolução com detector de ionização de chama) e HRGC-MS cromatografia gasosa de alta resolução acoplada à espectrometria de massas). Foram identificados fenilpropanóides e sesquiterpenos, estes últimos em pequenas quantidades, representando cerca de 97% da composição total da fração volátil. O principal componente, 2-fenilnitroetano, corresponde a 71,12% do extrato obtido por SFE.

Unitermos: *Aniba canelilla* (H.B.K.) Mez., Lauraceae, SFE-CO$_2$, HRGC-FID, HRGC-MS, fenilpropanóides, sesquiterpenóides.

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