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# *Aniba canelilla* (H.B.K.) Mez Essential Oil: Analysis of Chemical Constituents, Fungistatic Properties

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**ABSTRACT:** The composition of the essential oil and of the hexane extract of the stem bark of *Aniba canelilla* was analyzed by means of GC/MS, MS and NMR spectroscopy. In addition to confirming the presence of some previously identified constituents such as 1-nitro-2-phenylethane, safrole and eugenol, this study allowed us to identify β-sitosterol, α-pinene, β-pinene, benzaldehyde, phenylacetaldehyde, methoxy eugenol, methyl eugenol, and (–)-selin-11-en-4α-ol. 1-Nitro-2-phenylethane was found to exhibit a high toxicity towards yeasts especially *Candida albicans*. The $LD_{50}$ of a petroleum ether extract from this plant was determined to be greater than 800 mg/kg for BALB/c mice.

**KEY WORD INDEX:** *Aniba canelilla*, Lauraceae, essential oil composition, 1-nitro-2-phenylethane, (–)-selin 11-en-4α-ol, fungistatic activity, $LD_{50}$.

**INTRODUCTION:** *Aniba canelilla* (Lauraceae) constitutes with *A. ferrea* a particular group among the 41 species of the genus *Aniba* (1). This plant, which is endemic to South America, is a large tree. It possesses a stem bark which is locally chewed by children as a confection. According to the Chimane indians, bark infusions have been used to stop diarrhoea, to treat headaches and as a febrifuge. The wood which is resistant against parasitic attacks, is locally used to build pirogues (2,3).

The leishmanicidal activity of a petroleum ether extract obtained from the stem bark was first demonstrated in vitro during a preliminary screening. However this result was not confirmed in vivo on BALB/c mice infected with *Leishmania amazonenzis* (3).

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The alkaloids of *A. canelilla* have been the subject of previous study. Twenty-one isoquinoline derivatives have been isolated, twelve of which were identified as new benzyl tetrahydroisoquinolines or new tetrahydroprotoberberines (4).

**EXPERIMENTAL:** ***Plant Material***—The stem bark of *A. canelilla* was collected in Fatima de Chimanes (Beni department, Bolivia) in 1988. A voucher specimen (reference A.F. 855) has been deposited at the National Herbarium of Bolivia in La Paz.

***Oil and Extraction Isolation***—*Hydrodistillation:* Distillation of stem bark powder was carried out in a 1 L apparatus approved by French Pharmacopoeia (IX); the operating conditions were: load/water ratio: 10%, hydrodistillation time: 3 h, the distillation water was saturated with xylene and the oil was collected in 1 mL of xylene.

*Solvent extraction:* One kg of powdered stem bark was extracted with hexane (6 L) in a Soxhlet apparatus for 20 h. The solvent was then evaporated under reduced pressure.

The hexane extract (23.5 g) was column chromatographed over Si gel. Elution was done with hexane gradually enriched with ethyl acetate. Monitoring by TLC enabled similar fractions to be combined. Final purifications were obtained by preparative TLC on Si gel plates. Compounds were identified by spectral analysis (UV, IR, HRMS, $^{1}H$ and $^{13}C$-NMR).

***Analytic Techniques***—*Analysis of the essential oil:* GC/MS (electron impact) analysis were performed on a Delsi Nermag instrument (GC DN 200-Automass 120) equipped with a polar SGE 50 m x 0.32 mm BP-20 capillary column (0.25 µm film thickness). The operating conditions were: injector and detector temperature 240°C, oven programming: 60°-250°C (4°C/min) with helium as carrier gas.

GC analysis and quantification were performed on a GC3000 Varian gas chromatograph equipped with a nonpolar J&W 30 m x 0.25 mm DB-1 capillary column (0.25 µm film thickness). The operating conditions were: injector and detector temperature 240°C, oven programming: 50°-210°C (2°C/min), detector: FID and carrier gas: nitrogen. Components were identified by comparison of their mass spectra and retention indices with those already reported and by co-injection of authentic samples.

*Spectral analysis of the constituents of the hexane extract:* The constituents of the hexane extract were analyzed by means of mass spectrometry and $^{1}H$-NMR spectroscopy (270 MHz).

***Lethal Doses***—$LD_{50}$ was evaluated on BALB/c mice, supplied by the Charles River Breeding Laboratory. Mice weighing 18-20 g were eight weeks old when bioassays were initiated. Experimental doses were 12.5, 25, 50, 100, 200, 400 and 800 mg/kg. For each concentration, six mice were treated by intraperitoneal injection. The $LD_{50}$ was checked after 0, 24, 48 and 72 h.

***Microbiological Techniques***—Fungistatic properties of the 1-nitro-2-phenylethane were assayed against various clinical strains identified in our hospital laboratory, and belonging to the yeast group (*Candida albicans, C. tropicalis, C. parapsilosis*) or to the genus *Aspergillus* (*A. fumigatus*). Fungal suspensions in Casitone liquid medium for yeasts, or in Sabouraud liquid medium for *A. fumigatus*, were distributed in microtiter plates (200 µL per well) and incubated in the presence of 5 µL of serial two-fold dilutions of this compound in dimethyl sulfoxide. After incubation at 37°C for 24 h, the minimal inhibitory concentration (MIC) was estimated.

**Table I. Composition of the oil and hexane extract of the stem bark of *Aniba canelilla***

| Components | Hexane extract (mg) | Essential oil | |
|---|---|---|---|
| | | Percentage | RI |
| β-sitosterol | 200 | - | - |
| methoxy eugenol | 20 | - | - |
| benzaldehyde | - | 0.10 | 926 |
| α-pinene | - | 0.25 | 928 |
| β-pinene | - | 0.15 | 967 |
| phenylacetaldehyde | - | 0.15 | 1006 |
| unknown | - | 0.10 | 1086 |
| unknown | - | 0.10 | 1110 |
| α-terpineol | - | t | 1168 |
| 1-nitro-2-phenylethane | 18350 | 89.80 | 1255 |
| safrole | 1000 | 2.60 | 1263 |
| eugenol | 350 | 1.10 | 1325 |
| methyl eugenol | - | 2.90 | 1369 |
| selin-11-en-4α-ol | 50 | 1.00 | 1628 |

RI = retention indice on DB-1 column
t = trace

**RESULTS AND DISCUSSION:** As described by Gottlieb et al. (5) and Alpandé de Morais et al. (6) in previous studies of a Brazilian oil of *A. canelilla*, 1-nitro-2-phenylethane was found to be the major product. The NMR spectrum ($CDCl_3$) of this compound exhibited a multiplet centered at δ7.26 ppm corresponding to the five aromatic protons. Two triplets ($J$=7.5 Hz) were located at δ3.29 ppm for the methylenic protons in α position to the aromatic ring and at δ4.59 ppm for the methylenic protons in α position to the nitro group.

A sesquiterpene alcohol was also isolated from the hexane extract. HRMS gave a molecular ion at m/z 222.1977 which corresponded to the molecular formula $C_{15}H_{26}O$ (calculated 222.1983). Important features of the $^1$H-NMR spectrum ($CDCl_3$) were the methyl resonances at δ0.89 ppm (Me-10), 1.11 (Me-4) and 1.75 ppm (Me-11) while the exomethylene protons appeared as a multiplet centered at δ4.70 ppm. These data were compared with those already reported for eight synthetic diastereoisomers of eudesm-11-en-4-ol (7), and suggested this sesquiterpene to be (–)-selin-11-en-4α-ol. Confirmation was obtained by recording the NMR spectrum in deuteriated pyridine; in such conditions, a downfield shift (+0.15 ppm) of the Me-4 was observed. This feature is diagnostic of the 1,3-diaxial position of Me-4 and Me-10 (8), and as expected a reciprocal nOe was observed between these two methyl groups.

The chemical study of the stem bark oil was then undertaken (Table I). GC/MS chromatography, performed on two column types, allowed to identify the same components, except for β-sitosterol and methoxy eugenol. Six other compounds were also identified: three olefinic monoterpenes (α-pinene, β-pinene, α-terpineol) and three aromatic derivatives (benzaldehyde, phenylacetaldehyde and methyl eugenol). Mass spectrum of 1-nitro-2-phenylethane is characterized by a weak molecular ion at m/z 151 (<1%), and significant

**Table II. Fungistatic activities of 1-nitro-2-phenylethane**

| Species | Number of strains tested | MIC (µg/mL) |
|---|---|---|
| *C. albicans* | 10 | 170 ± 48 |
| *C. parapsilosis* | 5 | 360 ± 90 |
| *C. tropicalis* | 5 | 720 ± 180 |
| *A. fumigatus* | 10 | 1500 ± 280 |

fragments at m/z 105 and m/z 77 corresponding to the loss of the nitro group [M-46] and of the aliphatic nitro side chain [M-74] respectively. Identity of selin-11-en-4α-ol was established by co-injection of pure reference sample obtained from the hexane extract. Comparison of its mass spectrum with the one described by Kesselmans et al. (7) showed many similarities: the ratios between the peaks at m/z 135 and 125 and between m/z 135 and 151 were superior to 10; the ratio between the peaks at m/z 123 and 125 was near 5. However, the 1.3 ratio instead of 1.9 between the peaks at m/z 135 and m/z 137 would not have allowed us to distinguish selin-11-en-4α-ol from the two cis-fused eudesm-11-en-4-ol namely 7-epi-amiteol and 5-epi-paradisiol. The aforementioned NMR spectral analysis was then necessary.

Three compounds [1-nitro-2-phenylethane (72.6%), eugenol (0.8%), methyl eugenol (24.9%)] were previously identified in the Brazilian oil (5,6). This composition is in agreement with the one found in our study but the amounts of 1-nitro-2-phenylethane and methyl eugenol are quite different (see Table I). The biosynthesis of 1-nitro-2-phenylethane and methyl eugenol seems to be interdependant in *A. canelilla* since a high amount of one compound lowers the ratio of the other.

Natural nitro derivatives are fairly rare (9), and 1-nitro-2-phenylethane has been only described in a few species such as *Ocotea preciosa* (Lauraceae), *Dennetia tripetala* (Annonaceae) and *A. canelilla* (5,10,11). No biological assays have been reported for this compound. Considering the resistance of the wood to parasitic attacks, we decided to evaluate the fungistatic activity of the major component of the hexane extract. Some pure 1-nitro-2-phenylethane was thus tested against human pathogenic fungi: *Candida albicans, C. parapsilosis, C. tropicalis* and *Aspergillus fumigatus*. These assays (Table II) showed a high toxicity of the 1-nitro-2-phenylethane against yeasts, especially *C. albicans* (MIC: 170 µg/mL), whereas A. fumigatus was more resistant (MIC: 1500 µg/mL). The $LD_{50}$ was determined to be greater than 800 mg/kg for BALB/c mice after 24, 48 and 72 h.

On the basis of these results, it would be interesting to synthesize some derivates of 1-nitro-2-phenylethane in order to determine whether the biological activity of this original nitro compound could be improved.

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