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Chemical composition from volatile oil of the stem bark of *Guarea macrophylla* Vahl. ssp. *tuberculata* Vellozo (Meliaceae)

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ABSTRACT: The stem bark of *Guarea macrophylla* Vahl. ssp. *tuberculata* Vellozo (Meliaceae) was submitted to steam distillation and adsorption chromatographic separation. Seventeen sesquiterpenes, one diterpene and four fatty acids were identified using a combination of GC, GC–MS, ^{1}H- and ^{13}C-NMR.

KEY WORDS: *Guarea macrophylla* Vahl. ssp. *tuberculata* Vellozo; Meliaceae; volatile oil composition; ^{1}H- and ^{13}C-NMR; GC–MS; sesquiterpenes; manoyl oxide; fatty acids

Introduction

G. macrophylla ssp. *tuberculata* (Meliaceae) is a tree which grows in Brazil from Rio Grande do Sul to the Amazonas States.[1] In spite of the large number of chemical studies on Meliaceae species, there are only few works which report the occurrence of sesquiterpenes in members of this family.[2–5] In this work, we have characterized the main sesquiterpenes from the volatile oil from stem bark of *G. macrophylla* ssp. *tuberculata* by using a combination of four spectrometric techniques (GC, GC–MS, ^{1}H- and ^{13}C-NMR) after chromatographic separation. Other components, one diterpene and four fatty acids, were also identified.

Previous Work

G. macrophylla is an ornamental tree used in the shading of Brazilian pastures, which has been described as a toxic plant for the cattle; several cases of intoxication leading to death of the animals have been reported.[1] Chemical studies with species of *Guarea* describe the occurrence of meliacines, triterpenes, steroids, diterpenes, sesquiterpenes and coumarins.[3–10] Most of the work on species of the Family Meliaceae describe the isolation of polar compounds (limonoids). This is due mainly to the high biological potential of such compounds.[11] The intermediate polarity components, such as mono-oxygenated sesquiterpenes and diterpenes, have not been identified. The first work on *G. macrophylla* describes the chemical composition of the dichloromethane extract from leaves, from which one monoterpene, four sesquiterpenes, five diterpenes and one triterpene were characterized.[12] The presence of this class of compounds and the low number of sesquiterpenes identified in the Family Meliaceae led us to study the volatile oil of the same vegetable material. Using a combination of four spectrometric methods (GC, GC–MS, ^{1}H- and ^{13}C-NMR), it was possible, after chromatographic separation, to identify one monoterpene, 15 sesquiterpenes and eight diterpenes from the volatile oil extracted from the leaves.[13] This work, the second on the volatile oil from *G. macrophylla* ssp. *tuberculata*, describes the characterization of the volatile constituents from the stem bark. The crude oil was submitted to chromatographic separation and the fractions obtained were analysed by GC and GC–MS. The identification of the components was confirmed by analysing their NMR data.

Experimental

Plant Material

The bark wood of *G. macrophylla* ssp. *tuberculata* (Meliaceae) was collected in São Paulo city, São Paulo State, Brazil, on 3 November 1999. The plant material was compared by Professor Dr José Rubens Pirani to a

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voucher specimen at the Institute of Bioscience at Universidade de São Paulo, SP, Brazil. The fresh stem bark of *G. macrophylla* ssp. *tuberculata* (385 g) was submitted to hydrodistillation in a Clevenger-type apparatus for 4 h to yield 191.6 mg of the crude oil (0.05%).

Component Identification

The crude oil was submitted to a column chromatography using silica gel eluted with pentane, dichloromethane and dichloromethane/methanol (99:1). The chromatographic separation yielded 32 fractions (10 ml each), which were pooled together in 10 groups after analysing the GC chromatograms of each fraction. This procedure afforded pure compounds as well as mixtures. After analysis of the ^{1}H- and ^{13}C-NMR spectra as well as GC–MS of each of the fractions, it was possible to identify six sesquiterpenes and one diterpene. The fatty acids were identified from the mixture by GC, GC–MS, ^{1}H- and ^{13}C-NMR. The fractions with non-oxygenated sesquiterpenes were pooled and submitted to column chromatography with silica gel soaked with $AgNO_3$ (15%) and then eluted with pentane, dichloromethane and dichloromethane:acetone (99:1). Using the same methodology, 27 fractions were collected (7 ml each), which were pooled together in nine groups. These groups were analysed by a combination of the four techniques described above to identify 11 hydrocarbon sesquiterpenes.

NMR

NMR spectra were recorded at 125 MHz for ^{13}C and 500 MHz for ^{1}H (Bruker DRX-500) using $CDCl_3$ (Aldrich) as solvent and internal standard.

GC

A Hewlet-Packard 5890 series II (using helium as carrier gas) equipped with a FID detector and a capillary column HP-5, cross-linked 5% phenyl in 95% methyl silicone (30 m × 0.32 mm, film thickness 0.25 μm). An automatic injector (HP 7673) and an electronic integrator (HP 3396A) were used. The temperature programming was from 100 °C isothermal for 2 min, rising to 240 °C at 5 °C/min, then isothermal at 240 °C for 5 min. The (FID) injector and detector temperatures were 180 °C and 260 °C, respectively.

GC–MS

Analyses were carried out in an EI–MS 70 eV Hewllet-Packard HP-5973 coupled with a Hewlet-Packard HP-6890 with a DB-5 column (30 m × 0.25 mm, film thickness 0.25 μm), using the same temperature programming conditions as described above. The components were identified by comparison of their mass spectra with corresponding data of authentic compounds.

Table 1. Chemical composition of the essential oil from stem bark of *Guarea macrophylla* ssp. *tuberculata* (Meliaceae)

Compound	RR_t	RI*	Percentage
α-Cubebene	562	1343	6.0
α-Copaene	583	1362	0.8
β-Caryophyllene	625	1414	3.6
Aromadendrene	652	1434	2.6
α-Humulene	683	1438	1.2
allo-Aromadendrene	699	1445	1.4
cis-Bicyclogermacradiene	705	1461	1.9
Viridiflorene	732	1481	1.4
trans-Bicyclogermacradiene	748	1493	5.2
cis-Calamenene	770	1507	11.0
δ-Cadinene	782	1519	7.8
Ledol	849	1566	3.3
Globulol	857	1585	6.5
Viridiflorol	869	1593	5.0
cis-Cubenol	918	1616	1.6
Guai-6-en-10β-ol	924	1621	0.3
trans-Cubenol	937	1639	1.6
Hexadecanoic acid	1343	1913	2.7
Manoyl oxide	1386	1957	0.3
Linolenic acid	1543	2071	1.9
Linoleic acid	1552	2078	2.7
Stearic acid	1564	2086	1.9

* In DB-5.

Results and Discussion

Using this methodology, which is a combination of four techniques: GC, GC–MS, ^{1}H- and ^{13}C-NMR, after chromatographic separation, it was possible to identify 17 sesquiterpenes, one diterpene and four fatty acids, representing 70.7% of the total oil (Table 1). We can observe that it was composed mainly of sesquiterpenes, *cis*- calamenene being the major compound (11.0 %).

In the volatile oil from leaves of *G. macrophylla* ssp. *tuberculata*, the main components were characterized as sesquiterpenoids (35.9% of the crude oil) ledol and guai-6-en-10β-ol being the major compounds (13.9% and 17.3%, respectively).[13]

The retention time on GC and the mass spectrum of each component of the crude volatile oil did not show the presence of monoterpenes.[14] Since several sesquiterpenes and no monoterpenes have been identified in the volatile oil from the stem bark and leaves of *G. guidonia*[5,10] and from the stem bark of *G. cedrata*,[4] this observation might be characteristic of the *Guarea* species.

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