Pergamon

PII: S0031-9422(97)00762-0

LEAF ESSENTIAL OILS OF THREE PANAMANIAN *PIPER* SPECIES

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Abstract—The chemical composition of the leaf essential oils of *Piper arboreum*, *P. fimbriulatum* and *P. obliquum* were analyzed by a combination of GC-FID, GC-mass spectrometry and ^{13}C NMR methods. The main constituents found in the oil of *P. arboreum* were δ-cadinene, α-copaene and β-pinene; in the oil of *P. fimbriulatum*, β-caryophyllene and the oxygenated monoterpenes linalol and its acetate; and β-caryophyllene, spathulenol and caryophyllene oxide in that of *P. obliquum*. A few uncommon sesquiterpenes not previously characterized in *Piper* species were also identified, namely, 1,5-epoxysalvial-4(14)-ene in *P. obliquum*, as well as α- and β-selinene in *P. fimbriulatum*. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Piper* is distributed in tropical and sub-tropical regions of the world [1]. *Piper* species are widely used in folk medicine in Latin America and the West Indies and several chemical and biological investigations have been undertaken for their economic and medicinal interest [2–6]. We describe herein the chemical composition of three Panamanian *Piper* essential oils. No chemical data on *P. fimbriulatum* and *P. obliquum* have been found and only one short report on *P. arboreum* var. *latifolium* [7] has been published previously.

RESULTS AND DISCUSSION

The qualitative and quantitative analytical results of the three essential oils are shown in Tables 1–3; ca 80% of the constituents were identified in each sample. The unidentified parts of the oils were mainly constituted by a mixture of sesquiterpene hydrocarbons and oxygenated sesquiterpenes, whose individual percentages were always minor than 1.5%. The three oils did not contain significant amounts of phenylpropenoids, sesquiterpene hydrocarbons being the main group of constituents in all samples.

Early work on a variety of *P. arboreum* (var. *latifolium*) [7] enabled the identification of six major constituents in the oil, which accounted for 93.74% (main component germacrene D, 72.87%). In our case, we analyzed the minor constituents as well. We have identified 43 different compounds, which represent 82.6% of the oil. Sesquiterpene hydrocarbons were the main constituents, especially δ-cadinene (25.8%), α-copaene (7.4%) and germacrene D (5.3%), and some monoterpene hydrocarbons, like α- and β-pinene (4.3% and 6.6%, respectively) were also found. Our results are different from the oil analyzed by Machado *et al.* [7], suggesting that they probably are two different varieties. *Piper arboreum* Aublet is botanically related to *P. cordulatum* which has been used for skin infections [8].

On the other hand, the chemical composition of the leaf essential oil from *P. fimbriulatum* contained large amounts of sesquiterpene hydrocarbons, especially β-caryophyllene (11.3%) and germacrene D (12.8%). Several oxygenated monoterpenes, such as linalol and its acetate (5.3% of each) were also found. Analysis of the hydrocarbon fraction by a combination of ^{13}C NMR and GC-mass spectrometry enabled the identification of α- and β-selinene, and α- and δ-guaiene. Among *Piper* species, δ-guaiene (=α-bulnesene) has only been found previously in the essential oil of *P. nigrum* [9, 10].

The main components in the leaf essential oil of *P. obliquum* were sesquiterpenes, especially sesquiterpene

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Table 1. Composition of essential oil from leaves of *Piper arboreum* from Panama

Components	%	Identification methods*
Monoterpene hydrocarbons	16.9	
Tricyclene	0.3	GC-MS, RI_1, RI_2
α-Pinene	4.3	GC-MS, RI_1, RI_2, ^{13}C NMR
Camphene	0.1	GC-MS, RI_1, RI_2
Sabinene	4.0	GC-MS, RI_1, RI_2, ^{13}C NMR
β-Pinene	6.6	GC-MS, RI_1, RI_2, ^{13}C NMR
Myrcene	0.3	GC-MS, RI_1, RI_2
(*E*)-β-Ocimene	0.3	GC-MS, RI_1, RI_2
α-Terpinene	0.1	GC-MS, RI_1, RI_2
p-Cymene	0.1	GC-MS, RI_1, RI_2
Limonene	0.5	GC-MS, RI_1, RI_2
β-Phellandrene	0.2	GC-MS, RI_1, RI_2
Terpinolene	0.1	GC-MS, RI_2
Oxygenated monoterpenes	2.0	
Linalol	0.8	GC-MS, RI_1, RI_2
Terpinen-4-ol	0.2	GC-MS, RI_1, RI_2
α-Terpineol	0.1	GC-MS, RI_2
Nerol	0.1	GC-MS, RI_2
Linalyl acetate	0.8	GC-MS, RI_1, RI_2
Sesquiterpene hydrocarbons	54.7	
α-Cubebene	0.3	GC-MS, RI_1, RI_2
α-Copaene	7.4	GC-MS, RI_1, RI_2, ^{13}C NMR
β-Bourbonene	0.2	GC-MS, RI_1, RI_2
β-Elemene	1.3	GC-MS, RI_1, RI_2
β-Caryophyllene	4.4	GC-MS, RI_1, RI_2, ^{13}C NMR
α-Gurjunene	0.3	GC-MS, RI_1, RI_2
α-Humulene	0.3	GC-MS, RI_1
β-Farnesene†	1.6	GC-MS, RI_1, RI_2
γ-Muurolene	0.5	GC-MS, RI_1, RI_2
Germacrene D	5.3	GC-MS, RI_1, RI_2, ^{13}C NMR
Bicyclogermacrene	1.2	GC-MS, RI_1, RI_2
γ-Gurjunene	1.1	GC-MS, RI_2
α-Muurolene	4.2	GC-MS, RI_1, RI_2
δ-Cadinene	25.8	GC-MS, RI_1, RI_2, ^{13}C NMR
Cadina-1,4-diene	0.3	GC-MS, RI_1, RI_2
Calacorene†	0.5	GC-MS
Oxygenated sesquiterpenes	9.0	
6*E*-Nerolidol	5.2	GC-MS, RI_1, RI_2, ^{13}C NMR
Isocaryophyllene oxide	0.3	GC-MS, RI_1, RI_2
Viridiflorol	0.4	GC-MS, RI_1, RI_2
Cubenol	0.3	GC-MS, RI_1, RI_2
α-Cadinol	0.5	GC-MS, RI_1, RI_2
epi-Cubenol	0.6	GC-MS, RI_1, RI_2
Farnesyl ketone	0.8	GC-MS, RI_2
T-Cadinol	0.2	GC-MS, RI_1, RI_2
Spathulenol	0.7	GC-MS, RI_1
Total identified	82.6	

* RI_1: retention index on CW-20M; RI_2: retention index on SE-30.
† Exact isomer not identified.

Table 3. Composition of essential oil from leaves of *Piper obliquum* from Panama

Components	%	Identification methods*
Monoterpene hydrocarbons	0.6	
α-Pinene	0.2	GC-MS, RI_1, RI_2
β-Pinene	0.2	GC-MS, RI_1, RI_2
p-Cymene	0.2	GC-MS, RI_1, RI_2
Oxygenated monoterpenes	1.3	
Linalol	0.3	GC-MS, RI_1, RI_2
p-Cymen-8-ol	0.6	GC-MS, RI_1, RI_2
Linalyl acetate	0.4	GC-MS, RI_1, RI_2
Sesquiterpene hydrocarbons	53.9	
α-Cubebene	0.9	GC-MS, RI_1, RI_2
α-Copaene	5.6	GC-MS, RI_1, RI_2, ^{13}C NMR
β-Bourbonene	1.3	GC-MS, RI_1, RI_2
β-Elemene	1.2	GC-MS, RI_1, RI_2
β-Caryophyllene	27.6	GC-MS, RI_1, RI_2, ^{13}C NMR
α-Gurjunene	0.5	GC-MS, RI_1, RI_2
α-Humulene	2.3	GC-MS, RI_1, RI_2, ^{13}C NMR
Aromadendrene	1.2	GC-MS, RI_2, ^{13}C NMR
γ-Muurolene	0.6	GC-MS, RI_1, RI_2
Germacrene D	3.9	GC-MS, RI_1, RI_2, ^{13}C NMR
γ-Elemene	0.5	GC-MS, RI_1, RI_2
α-Muurolene	0.8	GC-MS, RI_1, RI_2
β-Bisabolene	4.5	GC-MS, RI_1, RI_2, ^{13}C NMR
γ-Cadinene	0.5	GC-MS, RI_1, RI_2
δ-Cadinene	1.8	GC-MS, RI_1, RI_2, ^{13}C NMR
Calacorene†	0.7	GC-MS
Oxygenated sesquiterpenes	24.1	
1,5-Epoxysalvial-4(14)-ene	2.6	GC-MS, ^{13}C NMR
Spathulenol	10.6	GC-MS, RI_1, RI_2, ^{13}C NMR
Globulol	0.7	GC-MS, RI_2
Caryophyllene oxide	8.3	GC-MS, RI_1, RI_2, ^{13}C NMR
Isocaryophyllene oxide	0.6	GC-MS, RI_2
10-*epi*-γ-Eudesmol	0.7	GC-MS, RI_2
T-Cadinol	0.8	GC-MS, RI_2
Cubenol	0.8	GC-MS, RI_2
Others	1.4	
Hexadecanoic acid	1.4	GC-MS, RI_1, RI_2
Total identified	81.3	

* RI_1: retention index on CW-20M; RI_2: retention index on SE-30.
† Exact isomer not identified.

hydrocarbons, such as β-caryophyllene (27.6%), α-copaene (5.6%) and β-bisabolene (4.5%), and spathulenol (10.6%) and caryophyllene oxide (8.3%) among the oxygenated sesquiterpenes. The presence of 1,5-epoxysalvial-4(14)-ene (2.6%), a sesquiterpene with a rare skeleton, which has not been found previously in *Piper* species, was established by ^{13}C NMR and GC-mass spectrometry. This compound was discovered for the first time in the essential oil of *Salvia sclarea* [11], and has been recently reported in the oils of *Baccharis dracunculifolia* [12] and several *Artabotrys* species [13].

Piper obliquum and the related species, *P. imperiale*,

P. fimbriulatum, *P. maxonii*, *P. cenocladum*, *P. gibbosum*, *P. euryphyllum* and *P. biseriatum* represent a group with unresolved taxonomic difficulties [14]. However, we have shown that *P. obliquum* and *P. fimbriulatum* are two different species, both from a quantitative and qualitative chemical point of view.

EXPERIMENTAL

Plant material

Leaves from wild plants were collected in Chepo (Panama) in the case of *P. arboreum* Aublet, and in

the National Park of Altos de Campana (Panama) for *P. fimbriulatum* CDC and *P. obliquum* Ruíz López & Pavón. Voucher specimens are included in the Herbarium FLORPAN (University of Panama) under numbers 2484 for *P. arboreum*, 2479 for *P. fimbriulatum* and 2480 for *P. obliquum*. Identification of plant material was made by Prof. Mireya Correa, Director of the Herbarium of the University of Panama (Panama). Leaves from the three species were hydrodistilled with a Clevenger-type apparatus to obtain essential oils in yields of 0.5, 1 and 0.8% for *P. arboreum*, *P. fimbriulatum* and *P. obliquum*, respectively.

Analysis of essential oils

Oils were analyzed by GC and GC-mass spectrometry using two fused silica capillary columns with different stationary phases. GC was performed using the following analytical conditions: columns (25 m × 0.2 mm i.d., 0.25 μm film) of Carbowax 20M (CW-20M) and methyl silicone 30 (SE-30), carrier gas He, flow rate 1 ml min^{-1}, split 1:60, inj. temp. 250°, oven temp. prog. from 80° to 250° at 4° min^{-1}, FID temp. 270°. GC-mass spectrometry were obtained using a computerized system coupled to a mass selective detector and a Supelcowax 10 capillary column (30 m × 0.2 mm i.d., 0.25 μm film) and the same SE-30 column as described above. Analytical conditions were: carrier gas He, flow rate 1 ml min^{-1}, split 1:60, inj. temp. 250°, interface temp. 290°, oven temp. prog. from 60° to 220° at 6° min^{-1}. Identification of components was made on the basis of their retention indices, with reference to a homologous series of fatty acid Me esters and their MS, which were compared with those of our library and with lit. data [15, 16]. Quantification of the components was performed on the basis of their GC peak areas, without corrections for response factors.

In addition, the identity of compounds comprising more than 1% in the three oils was also confirmed by ^{13}C NMR, using computer-aided analysis [17–20]. With the help of a laboratory-made software, the chemical shift of each carbon in the experimental spectrum was compared with those of the spectra of pure compounds listed in the laboratory spectral data bank containing around 350 spectra of mono-, sesqui- and diterpenes, and with lit. data. Each compound was identified unambiguously, taking into account the number of identified carbons, the number of overlapped signals, as well as the difference between chemical shifts of each resonance in the mixt. and in the reference. The ^{13}C NMR spectra of whole oils were recorded at 50 MHz, in CDCl$_3$, with all shifts referred to int. TMS, with the following parameters: pulse width (PW) 5.0 μs, acquisition time 1.3 s for 32 K data table with spectral width (SW) of 12,500 Hz (250 ppm), CPD mode-decoupling, digital resolution (0.763 Hz pt^{-1}). The number of accumulated scans was 10,000 for each sample of essential oil (ca 200 mg

of the oil in 2 ml CDCl$_3$). Exponential multiplication of the free induction decay with a line broadening of 1 Hz was used before Fourier transformation.

The essential oil of *P. fimbriulatum* was fractionated by means of CC over silica gel, eluting with hexane and petrol. The two frs obtained, corresponding mainly to hydrocarbons and oxygenated compounds, respectively, were analyzed again by ^{13}C NMR to confirm the identity of some minor compounds.

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