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Resumen

Composición química de los aceites esenciales de frutos de *B. simaruba* (L.) Sarg y resinas de tres especies de *Bursera*: *B. simaruba* (L.) Sarg, *B. glabra* Jack and *B. inversa* Daly. Las resinas de *B. simaruba*, *B. glabra* y *B. inversa* así como los aceites esenciales de los frutos de *B. simaruba* colectados en Mérida (Venezuela) fueron analizados por CG/EM. En las muestras de resina se identificaron 18, 7 y 10 compuestos de *B. simaruba*, *B. glabra* y *B. inversa*, respectivamente. Las resinas de *B. simaruba* y *B. glabra* se componen principalmente de monoterpenos mientras que los sesquiterpenos son predominantes en *B. inversa*. El componente mayoritario en las seis muestras analizadas de *B. simaruba* es el α -pineno (52-66 %); en tres de estas muestras el α -felandreno es el segundo componente mayoritario (24-31 %), mientras que para las otras tres es el germacreno D (11-18 %). Los componentes mayoritarios de la resina de *B. glabra* son limoneno (77,6 %) y *cis*-ocimeno (7,93 %), mientras que α -humuleno (27,7 %), β -cariofileno (22,1 %) y germacreno B (16,3 %) son los de *B. inversa*. Para los frutos de *B. simaruba* se identificaron 13 compuestos en los mesocarpos y 4 en los endocarpos. Los endocarpos se componen de ésteres metílicos de ácidos grasos (palmitato de metilo, oleato de metilo y linoleato de metilo), mientras que en los mesocarpos abundan los monoterpenos (sabineno (59,2 %) y terpinen-4-ol (12,3 %)).

Keywords: *Bursera*; monoterpenoides; resinas; sesquiterpenoides; aceites esenciales

Abstract

Resins from *B. simaruba*, *B. glabra* and *B. inversa* and essential oils from *B. simaruba* fruits collected in Mérida (Venezuela) were analyzed by GC-MS. The analysis of resins allowed the identification of 18, 7 and 10 compounds in *B. simaruba*, *B. glabra* and *B. inversa*, respectively. The resins of *B. simaruba* and *B. glabra* are dominated by monoterpenes while sesquiterpenes are the predominant components in *B. inversa*. The major component in the six *B. simaruba* samples analyzed was α -pinene (52-66 %); three of these samples have α -phellandrene (24-31 %) as other major compound, whereas germacrene D (11-18 %) is a major component of the other three samples. *B. glabra* is composed principally by limonene (77.6%) and *cis*-ocimene (7.93 %) whereas *B. inversa* contains α -humulene (27.7 %), β -caryophyllene (22.1 %) and germacrene B (16.3 %) as major components. On the other hand, 13 and 4 components were identified in endocarps and mesocarps of *B. simaruba* fruits. Endocarps are dominated by methyl esters of fatty acids (methyl oleate, methyl palmitate and methyl linoleate). Mesocarps are constituted by monoterpenes, the major components are sabinene (59.2 %) and terpinen-4-ol (12.3 %).

Keywords: *Bursera*; Monoterpenoids; Resins; Sesquiterpenoids; Essential oils

Introduction

Burseraceae family is distributed in tropical and subtropical regions around the world. The taxonomic classification of Burseraceae has been a long and unsatisfactory process; several plant characteristic (flower, fruit and seed anatomies, germination, etc.) has been used as basis to clarify the subdivision of this family^{1,2}. Actually, Burseraceae family is formed by 18 genres and more than 600 species; nine of these genres are only found in American continent³.

Bursera is one of Burseraceae genus found only in American continent and it contains more than 100 species distributed from the southern United State to Peru and the Caribbean². Actually, the genus is divided in three subgenus: *Bursera*, *Elaphrium* and *Buntingia*⁴. In Venezuela, there are reported 6 species of *Bursera*, 3 of them are endemic of northern South America and Caribbean.

Traditionally, species of *Bursera* genus has been used by some populations in the treatment of several illness and

symptoms, such as headache, toothache, uterine and urinary diseases, fever, rheumatism, bronchitis, eczema, etc^{3,5-7}.

Species belonging to this genus are usually rich in essential oils and resins which are usually composed by terpenoids⁸. Regarding the diverse reports for the chemical composition of essential oils and resins from *Bursera* species, it is found a significant variation in number, identity and individual relative amounts of terpenoids described in the analyzed species even in populations of the same species.

As part of our phytochemical study of *Bursera* genus, three species found in Mérida state (Venezuela), *B. glabra* Jacq., *B. inversa* Daly and *B. simaruba* L. Sarg., were studied. In this article we describe the identification by GC-MS of compounds presents in resins from the three selected species and the chemical composition of essential oil of fruits from *B. simaruba* L. Sarg.

Experimental

General experimental procedures

The GC-MS analysis were carried out on a Hewlett Packard GC-MS system, Model 5973, fitted with a 30 m long, cross-linked 5 % phenylmethyl siloxane (HP-5MS, Hewlett Packard, USA) fused-silica column (0.25 mm, film thickness 0.25 μ m). Source temperature 230 °C; quadrupole temperature 150 °C; carrier gas helium, adjusted to a linear velocity of 34 m/s; ionization energy, 70 eV; scan range 40-500 amu; 3.9 scans/s. The injected volume was 1.0 μ L. The initial oven temperature was 100 °C and then raised to 300 °C at 5 °C/min for resins, essential oils and fatty acid methyl esters. The final temperature was maintained for 20 min. A Hewlett-Packard ALS injector was used with split ratio 1:100. Identification of the resin components was supported on the Wiley MS Data Library (6th edition), followed by comparison of MS data with published literature^{9,10}. Kováts retention indices (KI) were determined relative to the retention times of a series of n-paraffin hydrocarbons (C₇-C₂₂)^{9,10}.

Plant material

The samples of three *Bursera* species were collected in Mérida (Venezuela):

B. simaruba (L.) Sarg.:

Location A: collected on July 2015 at 1100 m.a.s.l. in the xerophytic zone between Ejido and Las Gonzales, in the highway to Los Guáimaro (Mérida State). Coordinates: 8°32'4.7" N; 71°15'34.2" W. Samples of two different specimens were collected (A1 and A2)

Location B: collected on July 2015 at 1050 m.a.s.l. in the Botanic Garden "Ing. Carlos Liscano" (San Juan de Lagunillas, Mérida State). Coordinates: 08°30'38.0" N; 71°21'98.5" W. Samples of three different specimens were collected (B1, B2 and B3)

Location C: collected on October 2015 at 710 m.a.s.l. in rural highway between Estanques and Mesa Bolívar (Mérida State). Coordinates: 8°27'52" N; 71°32'44" W.

B. glabra (Jacq.): Collected in blooming period on February 2016 at 1900 m.a.s.l. in highway to El Morro, between El Morro's bridge and the detour to El Mocaz (Mérida State). Coordinates: 8°27'00.7" N; 71°11'09.1" W.

B. inversa Daly: Collected in fruit period on February 2016 at 36 m.a.s.l. in El Vigía (Mérida state), Bolívar Avenue, in the way to Santa Bárbara del Zulia. Coordinates: 8°38'24.1" N; 71°40'3.9" W.

The samples identification was carried on by professors José Guevara and Enrique Gámez from the Faculty of Forest and Environmental Sciences (Universidad de Los Andes), professor Mercedes Castro from Agronomy Faculty (Universidad Central de Venezuela) and staff of the Botanic Garden "Ing. Carlos Liscano".

Resin samples collection and treatment

To collect resin samples, a cut was made over the plant, approximately at one meter of height over the ground, with a sterile surgical knife to remove a bark piece of 50 cm² and 2-4 mm of thickness. Then, after 2-3 min, the resin started to appear. The resin was collected in opaque test tubes and stored at low temperatures until the analysis to avoid the degradation and aging of the resin.

For the GC-MS analysis, around 5 mg of resin was solubilized in 10 ml of hexane and few drops of dichloromethane, then it was shake until the resin was completed dissolved. The injected volume was 1.0 μ L.

Fruit samples collection and treatment

B. simaruba fruits (200 g) were cut to separate the fleshy mesocarp (160 g) and the osseous endocarp (40 g).

Essential oil from mesocarps: The mesocarp was cut in small pieces and then was submitted to hydrodistillation by 3 hours with a Clevenger trap to obtain 4 ml of essential oil. This was dried with Na₂SO₄ anhydride and kept under atmosphere of N₂ at 4 °C.

For the GC-MS analysis the essential oil was solubilized in hexane. The injected volume was 1.0 μ L.

Fatty acids from endocarps: The endocarp was dried at 40 °C by 24 hours. Then it was powdered and submitted to Soxhlet extraction with hexane at 65 °C by 48 hours to obtain a yellowish oil. This oil was extracted with NaOH 10% to yield 410 mg of a mixture of fatty acids. After, the mixture of fatty acids was treated with diazomethane to obtain the methyl esters.

For the GC-MS analysis essential oil was solubilized in ethyl ether. The injected volume was 1.0 μ L.

Results and discussion

The resin samples obtained from the three *Bursera* species were analyzed by GC-MS in order to identify the volatile compounds of the samples. The GC-MS analysis yields a total of twenty-nine well-known monoterpenes and sesquiterpenes. The compounds were identified by comparison of spectrometry data with literature values. The table 1 shows the compounds and its relative amount (weight percent, % w/w) found in every resin sample.

Results in table 1 showed significant difference in the compounds found in resins from *B. inversa*, *B. glabra* and *B. simaruba*. The difference in its chemical composition could be an indicator that confirm the subdivision of *Bursera* genus in three subgenus proposed by Castro-Laporte⁴, where *B. inversa*, *B. glabra* and *B. simaruba* are classified inside the *Buntingia*, *Elaphrium* and *Bursera* subgenus, respectively.

In *B. simaruba* resins were identified eighteen components in the six samples analyzed which correspond to 90.3-95.7 % of the total resins. These resins are dominated by monoterpenes. All resin samples has a higher amount of monoterpenes (59-90 %) than sesquiterpenes (3-33 %) where the α -pinene is the major compound (52-66 %). The results shows that resin samples of *B. simaruba* could be separated in two groups based in the proportion of monoterpenes and sesquiterpenes found in the samples. One group has a monoterpene/sesquiterpene ratio of approximately 90/3 and the other a 60/30 ratio. Samples having ~90 % of monoterpenes (B2, B3 and C) contain a significant amount of monoterpene α -phellandrene (24-31 %) which was not observed in other group. This last one group showed an increase in the amount of some sesquiterpenes as germacrene D (11-18 %), α -copaene (5-6 %) in A1 and A2, β -bourbonene (4.5 %) in A1, α -cubebene (5.1 %) in A2 and β -caryophyllene (14.6 %) in B1.

Table 1: Percentage composition (% w/w) of resins from *B. simaruba*, *B. glabra* and *B. inversa*.

Compound	Specie	<i>B. simaruba</i>						<i>B. glabra</i>	<i>B. inversa</i>
	KI	A1	A2	B1	B2	B3	C		
α -Pinene	933	52.9	52.2	66.7	55.1	54.4	62.1	-	-
β -Pinene	967	9.0	7.8	-	-	2.5	2.0	-	-
β -Myrcene	976	-	-	-	-	2.0	1.8	-	-
α -Phellandrene	988	-	-	-	31.1	30.5	24.6	1.8	-
<i>p</i> -Cymene	1005	-	-	-	0.2	-	-	-	-
Limonene	1008	-	-	-	-	-	-	77.6	1.0
<i>cis</i> -Ocimene	1017	-	-	-	-	-	-	7.9	-
<i>trans</i> -Carveol	1216	-	-	-	-	-	-	1.9	-
<i>L</i> -Carvone	1245	-	-	-	-	-	-	1.4	-
α -Bornyl acetate	1290	-	-	-	0.4	0.5	-	-	-
α -Cubebene	1354	0.5	5.1	-	-	-	-	-	-
α -Copaene	1379	5.2	5.9	2.4	0.5	0.5	-	-	1.9
β -Bourbonene	1388	4.5	0.5	-	-	-	-	-	-
β -Cubebene	1409	2.2	-	-	0.2	-	-	-	-
<i>cis</i> -Caryophyllene	1421	-	-	-	-	-	-	1.7	-
β -Caryophyllene	1423	3.9	-	14.6	-	-	-	-	22.1
Germacrene D	1474	12.6	18.1	11.5	2.3	2.5	3.5	-	1.3
α -Humulene	1460	0.9	1.1	-	-	-	-	-	27.7
<i>neo</i> -Allocymene	1467	0.5	-	-	-	-	-	-	-
α -Amorphene	1482	0.3	-	-	-	-	-	-	1.0
β -Selinene	1492	-	-	-	-	-	-	-	2.7
α -Selinene	6500	-	-	-	-	-	-	-	1.0
Bicyclogermacrene	1503	0.8	0.8	-	0.2	0.3	-	-	3.6
δ -Cadinene	1527	1.7	1.9	-	0.4	0.5	-	-	-
Germacrene B	1557	-	-	-	-	-	-	-	16.3
Caryophyllene oxide	1580	-	-	-	-	-	-	1.9	-
Palmitic acid*	1972	0.8	-	-	-	-	-	-	-
% Monoterpenes		62.3	60.0	66.7	86.8	89.8	90.5	90.5	1.0
% Sesquiterpenes		33.4	33.3	28.5	3.5	3.7	3.5	3.6	86.4
% knowns		95.7	93.3	95.2	90.3	93.5	94.0	94.1	87.4
% unknowns		4.3	6.7	4.8	9.7	6.5	6.0	5.9	12.6

* Quantified as methyl esters; KI = Kovats retention index

There are previous phytochemical analysis of essentials oils from *B. fagaroides*¹¹, *B. hollicki*¹², *B. lunani*¹³, *B. morelensis*¹⁴ and *B. schlechtendalii*⁸ species, which belongs to *Bursera* subgenus. These studies showed that monoterpenes α -pinene and α -phellandrene are usually major compounds of essentials oils from species classified in this subgenus. The comparison between obtained results for resin of *B. simaruba* and the chemical composition of leaves, fruits and bark essential oils from the same plant found in Jamaica¹⁵ showed that essential oils of both species have a similar composition with α -pinene as the major component. The mainly difference between Venezuelan and Jamaican species is the presence of germacrene D in all *B. simaruba* samples collected in Mérida (Venezuela). Since the resin composition from Jamaican and Venezuelan species of *B. simaruba* are similar, it seems that identity of resin components is principally inherent to the specie and external factors (as region, climate, soil, etc.) could affect the relative amount of these components, which could explain the presence of germacrene D in Venezuelan species. However, it is necessary a detailed research under controlled conditions to determine the accurate causes of the observed chemical composition variations.

Resin from *B. glabra* is constituted by eight components which correspond to 94.1 % of total resin. This resin is composed mainly by monoterpenes (90.5 %) with a proportion of monoterpenes and sesquiterpenes similar to samples B1, B2 and C of *B. simaruba*. However, for the *B. glabra* resin the main compounds are limonene and *cis*-ocymene (77.6 % and 7.9 %, respectively) and the α -pinene is not reported. Despite of *B. glabra* and *B. simaruba* resins have a similar monoterpenes/sesquiterpenes proportion, the chemical composition of *B. glabra* resin is very different to *B. simaruba* resins, and α -phellandrene is the only one compound in common for the resins of both species.

The analysis of the volatile compounds for other species of *Elaphrium* subgenus revealed the monoterpenes limonene and *cis*-ocimene as major compounds in the essentials oils and resins from *B. graveolens*¹⁶⁻¹⁸ and *B. tomentosa*¹⁹.

The specie *B. inversa* is the only specie classified inside *Buntingia* subgenus and there is a great difference in chemical composition between *B. inversa* resin and the resins from the other species studied in this work. In *B. inversa* resin, 10 components were identified which constituted 87.4 % of total resin. The predominant compounds are sesquiterpenes and represent the 86.4 % of the composition, with a proportion of 86/1 of sesquiterpenes/monoterpenes. The principal compounds are α -humulene (27.7 %), β -caryophyllene (22.1 %) and germacrene B (16.3 %).

The comparison between *B. inversa* resins and *B. glabra* and *B. simaruba* resins showed that *B. inversa* has some compounds in common with *B. glabra* and *B. simaruba*. However, the main compounds found in resin of *B. glabra* and *B.*

simaruba were not located in *B. inversa* resin or their relatives amounts are no substantial (<5 %).

The *B. simaruba* fruits were separated in mesocarps and endocarps in order to determinate their essential oil chemical compositions. Results are listed in Table 2. The analysis allowed the identification of thirteen compounds in mesocarps and four components in endocarps. The components identified in mesocarps and endocarps correspond to 99.5 % and 87.3 % of total oils, respectively. The endocarps are composed principally, as it was expected, by fatty acids (determined as methyl esters) with the linoleic acid (28.5 %), palmitic acid (25.2 %) and oleic acid (24.7 %) as major components while for the mesocarps its essential oil is composed only by monoterpenes and the major compounds are sabinene (59.2 %), terpinen-4-ol (12.3 %) and α -pinene (7.6 %).

The composition of the mesocarp essential oil is very similar in terpenoid identity to essential oil from Jamaican *B. simaruba* fruits¹⁵. However, the relative amounts and major components are slightly different. In Jamaican fruits, the major compound are α -pinene (28 %), β -pinene (24 %) and terpinen-4-ol (13 %), whereas the sabinene is present in a minor amount (8 %). This difference in the amount relative of each compound could be explained if, as mentioned above, the environment of the analyzed specie has influence in relative amount of secondary metabolites produced by the plant.

Table 2: Percentage composition (% w/w) of fruits from *B. simaruba*.

Compound	KI	Location A	
		Mesocarps	Endocarps
α -Thujene	919	3.0	-
α -Pinene	926	7.6	-
Sabinene	960	59.2	-
β -Pinene	962	2.2	-
Myrcene	970	2.7	-
α -Terpinene	991	2.8	-
<i>o</i> -Cymene	997	0.5	-
β -Phellandrene	1001	2.1	-
γ -Terpinene	1038	5.1	-
Terpinolene	1074	1.2	-
<i>p</i> -Menth-2-en-1-ol	1131	0.4	-
Terpinen-4-ol	1171	12.3	-
Palmitic acid*	1179	-	25.2
α -Terpineol	1182	0.4	-
Linoleic acid*	1232	-	28.5
Oleic acid*	1233	-	24.7
Stearic acid*	1240	-	8.8
% knowns		99.5	87.3
% unknowns		0.5	12.8

* Quantified as methyl esters; KI = Kovats retention index

Conclusions

The analysis of resins from *B. simaruba*, *B. glabra* and *B. inversa* and essential oil of *B. simaruba* fruits allowed a better knowledge of the chemical composition of this species. Since there are not previous reports for *B. glabra* and *B. inversa* resins, the identification of the resin components is a great contribution to increase the phytochemical knowledge of *Bursera* species.

Results of *B. inversa* resins are remarkable due to higher amount of sesquiterpenes. The other studied species showed a major proportion of monoterpenes than sesquiterpenes.

Since results from chemical analysis of *B. simaruba* and *B. glabra* resins and essential oil of *B. simaruba* fruits are whole consistent with the data reported to other species belonging to the same subgenus, a detailed research of the secondary metabolites in *Bursera* species could show the presence of chemotaxonomic markers specific for each *Bursera* species. In addition, further studies under controlled conditions will allow determine a relationship between environment and relative amount of essential oil components.

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