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Composition of the essential oils from leaves and fruits of three *Hedyosmum* species from Costa Rica

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ABSTRACT: Essential oils obtained by hydrodistillation from leaves and fruits of *Hedyosmum mexicanum* Cordemoy, leaves of *H. bonplandianum* Kunth and leaves of *H. costaricensis* C.E. Wood from Costa Rica were analyzed by GC-FID and GC-MS. GC-IR and ^{13}C-NMR analysis were performed to improve the identification of some compounds. Monoterpene hydrocarbons, especially sabinene, and oxygen-containing sesquiterpenes, mainly furanodiene, were the major constituents found in the oils of *H. mexicanum*. Monoterpene hydrocarbons were also the main group of components in the oil of *H. bonplandianum*, whereas the oil of *H. costaricensis* was mainly constituted by sesquiterpene hydrocarbons, especially germacrene-D. ^{13}C-NMR analysis of the latter oil allowed us to identify the compound 1β,6α-dihydroxy-4(14)-eudesmene. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: *Hedyosmum mexicanum* Cordemoy; *Hedyosmum bonplandianum* Kunth; *Hedyosmum costaricensis* C.E. Wood; Chloranthaceae; essential oil; GC-MS; ^{13}C-NMR; furanosesquiterpenes; 1β,6α-dihydroxy-4(14)-eudesmene.

Introduction

The Chloranthaceae family mainly includes Asian plants grouped in four genera: *Chloranthus*, *Sarcandra*, *Ascarina* and *Hedyosmum*.[1] Only the last genus, which is represented by about 40 species of trees and shrubs growing in the mountains, is found in America.[2] The present work deals with the composition of the essential oils from leaves and fruits of *Hedyosmum mexicanum* Cordemoy, leaves of *H. bonplandianum* Kunth and leaves of *H. costaricensis* C.E. Wood from Costa Rica. No references concerning the chemical composition of the essential oils from these species were found in the literature.

Experimental

Plant Material

Leaves and fruits of *H. mexicanum* were collected in El Empalme, Cartago (Costa Rica) in March 1995, and leaves of *H. costaricensis* and of *H. bonplandianum* in La Balsa de S. Ramón, Alajuela (Costa Rica) in September 1996. Plant material was identified by Luis J. Poveda (Escuela de Ciencias Ambientales, UNA, Heredia) and by J. Gómez-Laurito (Escuela de Biología, UCR). Voucher specimens were deposited in the Herbarium of the University of Costa Rica under members USJ 56542 for *H. mexicanum*, USJ 52028 for *H. costaricensis* and USJ 52030 for *H. bonplandianum*.

Isolation and Analysis of Essential Oils

Air-dried leaves and fruits of *H. mexicanum* were submitted to hydrodistillation for 1.5 h affording essential oil yields of 0.40% (v/w) and 0.60% (v/w), respectively. Hydrodistillation (1.5 h) of fresh leaves of *H. bonplandianum* and *H. costaricensis* revealed oil contents of 0.06% and 0.10%, respectively. Analysis of the volatile oils was carried out by GC and GC-MS. GC analyses were performed on a Hewlett Packard 6890 gas chromatograph equipped with an FID, using two fused-silica columns with different stationary phases. Analytical conditions were: columns, Supelcowax 10™ (30 m × 0.2 mm i.d., 0.25 μm film thickness) and methylsilicone (SE-30) (30 m × 0.2 mm i.d., 0.25 μm film thickness); carrier gas, He (1 ml/min); split ratio, 1 : 60;

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injector temperature, 250°C; oven temperature programme, 60°C to 250°C at 4°C/min; detector temperature, 270°C. MS were obtained with a Hewlett-Packard 5890 gas chromatograph coupled to a mass selective detector Hewlett-Packard 5971A, using the same columns as above. Analytical conditions were: carrier gas, He (1 ml/min); split ratio, 1 : 60; injector temperature, 250°C; interface temperature, 290°C; oven temperature programme, 60–220°C at 4°C/min; ionization voltage, 70 eV; scan range, 35–400 u. Identification of components was achieved from their GC retention indices in the two stationary phases, relative to a homologous series of fatty acid methyl esters, and by comparison of fragmentation patterns in mass spectra with those stored on our own database and with literature data.[3]

Computer-aided analysis of the ^{13}C-NMR spectra of the total essential oils[4–6] was also performed to confirm the identity of the components with a percentage higher than 1%. NMR spectra were recorded on a Bruker AC 200 Fourier Transform spectrometer operating at 50.323 MHz for ^{13}C and equipped with 10 mm (or 5 mm) probe, in deuterated chloroform, with all shifts referred to internal TMS. Other parameters were: pulse width, 5.0 μs (or 3.0 μs); flip angle, 45°; acq. time 1.3 s for 32 K data table with spectra width of 12 500 Hz (250 ppm); CPD mode decoupling; digital resolution, 0.763 Hz/pt. The number of accumulated scans was 5000–10 000 for each sample (200 mg [or 70 mg] of the oil in 2 ml [or 0.5 ml] $CDCl_3$).

When necessary, GC-FTIR was also used to improve the identification of some compounds such as furanodiene or furanoelemene. The IR spectra of these compounds were obtained using a GC-FTIR system equipped with a Supelcowax 10™ column (30 m × 0.2 mm i.d., 0.25 μm film thickness) under the following conditions: carrier gas, He (1 ml/min); oven temperature programme, 60–230°C at 4°C/min; injector temperature, 250°C; FTIR resolution, 8 cm^{-1}; transfer line temperature, 250°C; light pipe temperature, 250°C.

Results and Discussion

Essential Oils from Leaves and Fruits of *Hedyosmum mexicanum*

The results obtained in the analysis of the essential oils from the leaves and fruits of *H. mexicanum* are shown in Table 1. In total 39 different constituents were identified, meaning 86–96% of the total oils. Monoterpene hydrocarbons and some furanosesquiterpenes were shown to be the main groups of constituents in both oils, which appeared to be qualitatively different.

A large amount of monoterpene hydrocarbons, especially sabinene (28% in the leaf oil and 25% in the oil from the fruits) was found. Among the sesquiterpenes, furanodiene and furanoelemene (= curzerene) were identified on the basis of their GC-MS, GC-IR and ^{13}C-NMR data. Rearrangements of the latter compounds occurring during GC-FID analysis caused an important slope at the end of the chromatogram which hindered their quantification. These kind of compounds have previously been reported to be present in the Chloranthaceae[7] as well as in other species such as *Curcuma zeodaria*,[8] *Commiphora molmol*,[9] *Eugenia uniflora*,[10] and diverse marine soft-corals.[11] The co-occurrence of germacrane derivatives (B- and D-isomers) in both oils was also detected. A notable qualitative difference between both samples was the presence of the biogenetically related compounds menthone, isomenthone and pulegone in the essential oil from the leaves, which were absent in that from the fruits. Terpinen-4-ol, which amounted to 13% in the oil from the fruits, was scarcely present in the leaf oil (0.1%).

Essential Oil from the Leaves of *Hedyosmum costaricensis*

The analysis of the essential oil from the leaves of *H. costaricensis* allowed the identification of 29 components that represented 74% of the total oil (Table 2). Sesquiterpene hydrocarbons were the main group of compounds (63%), especially germacrene-D (32%), (*E*,*E*)-α-farnesene (8%), β-caryophyllene (6%) and β-bourbonene (6%). Among the monoterpene hydrocarbons, (*E*)-β-ocimene (2%) was the major constituent. The almost complete absence of oxygen-containing monoterpenes is remarkable; only linalol was found in a percentage of 0.7%. Among oxygen-containing sesquiterpenes, furane derivatives, some of them similar to those of the *H. mexicanum* oil, were detected in a low percentage (ca. 5%). ^{13}C-NMR analysis of the total essential oil allowed the identification of 1β,6α-dihydroxy-4(14)-eudesmene, the spectral data of which were in accordance with those previously given in the literature.[12] This compound has only been found before in *Artemisia eriopoda*[12] and *Ageratina glechonophylla*.[13]

Essential Oil from Leaves of *Hedyosmum bonplandianum*

A number of 51 components were identified in the essential oil of *H. bonplandianum* meaning 84% of the total oil (Table 2). Monoterpene hydrocarbons were shown to be the main fraction of the sample analyzed, followed by that of the sesquiterpene hydrocarbons. Among the former, sabinene (15%) and (*E*)-β-ocimene (11%) were the major constituents, while α-bisabolene (10%) and bicyclogermacrene (6%) were the main sesquiterpene hydrocarbons. Terpinen-4-ol (7%), linalol (3%) and

Table 1. Composition of the essential oils from leaves (L) and fruits (F) of *Hedyosmum mexicanum* Cordemoy

Component	*L* %	Identification method*	*F* %	Identification method**
Monoterpene hydrocarbons	**44.2**		**55.9**	
α-Thujene	0.7	GC-MS, RI_1, RI_2	2.2	GC-MS, RI_1, RI_2, ^{13}C-NMR
α-Pinene	2.1	GC-MS, RI_1, RI_2	3.4	GC-MS, RI_1, RI_2, ^{13}C-NMR
Camphene	0.7	GC-MS, RI_1, RI_2	0.8	GC-MS, RI_1, RI_2
Sabinene	28.0	GC-MS, RI_1, RI_2	24.6	GC-MS, RI_1, RI_2, ^{13}C-NMR
β-Pinene	4.6	GC-MS, RI_1, RI_2	8.0	GC-MS, RI_1, RI_2, ^{13}C-NMR
Myrcene	0.8	GC-MS, RI_1, RI_2	1.1	GC-MS, RI_1, RI_2, ^{13}C-NMR
α-Phellandrene	–		0.1	GC-MS, RI_1, RI_2
α-Terpinene	1.0	GC-MS, RI_1, RI_2	4.0	GC-MS, RI_1, RI_2, ^{13}C-NMR
Limonene	0.6	GC-MS, RI_1, RI_2	0.9	GC-MS, RI_1, RI_2
(*E*)-β-Ocimene	2.0	GC-MS, RI_1, RI_2	–	
γ-Terpinene	2.0	GC-MS, RI_1, RI_2	7.5	GC-MS, RI_1, RI_2, ^{13}C-NMR
p-Cymene	1.3	GC-MS, RI_1, RI_2	1.7	GC-MS, RI_1, RI_2, ^{13}C-NMR
Terpinolene	0.4	GC-MS, RI_1, RI_2	1.6	GC-MS, RI_1, RI_2, ^{13}C-NMR
Oxygen-containing monoterpenes	**7.2**		**19.4**	
1,8-Cineole	0.7	GC-MS, RI_1, RI_2	1.4	GC-MS, RI_1, RI_2
trans-Sabinene hydrate	–		0.7	GC-MS, RI_1, RI_2, ^{13}C-NMR
trans-Menthone	3.0	GC-MS	–	
Isomenthone	0.1	GC-MS	–	
Linalol	1.2	GC-MS, RI_1, RI_2	1.1	GC-MS, RI_1, RI_2, ^{13}C-NMR
Linalyl acetate	0.2	GC-MS, RI_1, RI_2	0.8	GC-MS, RI_1, RI_2
Terpinen-4-ol	0.1	GC-MS, RI_2	13.3	GC-MS, RI_1, RI_2, ^{13}C-NMR
Pulegone	1.4	GC-MS, RI_2	–	
α-Terpineol	0.1	GC-MS, RI_2	1.0	GC-MS, RI_1, RI_2, ^{13}C-NMR
Borneol	0.2	GC-MS, RI_1, RI_2	1.0	GC-MS, RI_1, RI_2, ^{13}C-NMR
Nerol	0.1	GC-MS, RI_1, RI_2	0.1	GC-MS, RI_2
Neryl acetate	0.1	GC-MS, RI_1, RI_2	–	
Sesquiterpene hydrocarbons	**11.9**		**6.2**	
β-Bourbonene	0.1	GC-MS, RI_1	–	
β-Elemene	1.4	GC-MS, RI_1, RI_2	0.8	GC-MS, RI_1, RI_2
β-Caryophyllene	0.7	GC-MS, RI_1, RI_2	0.5	GC-MS, RI_1, RI_2
α-Guaiene	–		0.3	GC-MS, RI_2
allo-Aromadendrene	–		0.1	GC-MS, RI_2
α-Humulene	0.2	GC-MS, RI_1	–	
γ-Muurolene	–		0.3	GC-MS, RI_2
Germacrene-D	1.0	GC-MS, RI_1, RI_2	0.2	GC-MS, RI_1, RI_2
Bicyclogermacrene	3.7	GC-MS, RI_1, RI_2	2.7	GC-MS, RI_1, RI_2, ^{13}C-NMR
α-Bisabolene	0.1	GC-MS, RI_1	0.1	GC-MS, RI_1
Germacrene-B†	4.7	GC-MS, RI_1	1.2	GC-MS, RI_1
Oxygen-containing sesquiterpenes	**22.4**		**14.1**	
Spathulenol	1.4	GC-MS, RI_1, RI_2	1.1	GC-MS, RI_1, RI_2, ^{13}C-NMR
Furanodiene‡	>20	GC-MS, RI_1, RI_2, GC-IR	>12	GC-MS, RI_1, RI_2, GC-IR, ^{13}C-NMR
Furanoelemene‡	>1	GC-MS, RI_1, RI_2	>1	GC-MS, RI_1, RI_2, ^{13}C-NMR
TOTAL	**85.7**		**95.6**	

* RI_1: Retention index in Supelcowax 10™.

** RI_2: Retention index in SE-30.

† Tentatively identified.

‡ Estimated percentages.

spathulenol (1%) were the only oxygen-containing compounds found in a percentage higher than 1%. Furanosesquiterpenes were not detected in this oil.

A comparison between the composition of the essential oils from the leaves of the three *Hedyosmum* species investigated showed both qualitative and quantitative differences. Monoterpene hydrocarbons were the main fraction of the leaf oils of *H. mexicanum* and *H. bonplandianum*, sabinene being the major constituent in both samples (28% and 15%, respectively). In contrast, the oil of *H. costaricensis* was characterized by a low percentage of monoterpenes, main constituent being germacrene-D (32%). An interesting group of compounds, found in a high percentage in the oil from *H. mexicanum* and in a lower percentage in the oil from *H. costaricensis*, were a series of furanosesquiterpenes, among which furanodiene and furanoelemene were identified.

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Table 2. Composition of the essential oils from leaves of *Hedyosmum costaricensis* C.E. Wood (HC) and *Hedyosmum bonplandianum* Kunth (HB)

Component	HC %	Identification method*	HB %	Identification method*
Monoterpene hydrocarbons	**3.8**		**39.7**	
α-Thujene	–		0.9	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
α-Pinene	0.1	GC-MS, RI$_1$, RI$_2$	2.5	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
Camphene	–		0.3	GC-MS, RI$_1$, RI$_2$
Sabinene	0.1	GC-MS, RI$_1$, RI$_2$	14.7	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
β-Pinene	0.1	GC-MS, RI$_1$, RI$_2$	3.5	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
Myrcene	0.1	GC-MS, RI$_1$, RI$_2$	0.4	GC-MS, RI$_1$, RI$_2$
α-Phellandrene	–		0.1	GC-MS, RI$_1$, RI$_2$
Δ3-Carene	†	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR	–	
α-Terpinene	–		1.8	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
Limonene	0.4	GC-MS, RI$_1$, RI$_2$	0.8	GC-MS, RI$_1$, RI$_2$
(*Z*)-β-Ocimene	–		1.8	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
(*E*)-β-Ocimene	2.3	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR	10.8	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
γ-Terpinene	0.4	GC-MS, RI$_1$, RI$_2$	0.8	GC-MS, RI$_1$, RI$_2$
p-Cymene	0.3	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR	0.4	GC-MS, RI$_1$, RI$_2$
Terpinolene	–		0.9	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
Oxygen-containing monoterpenes	**0.7**		**12.6**	
Linalol	0.7	GC-MS, RI$_1$, RI$_2$	3.0	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
Campholenal	–		0.4	GC-MS, RI$_1$, RI$_2$
Camphor	–		0.1	GC-MS, RI$_1$, RI$_2$
Isopulegol	–		0.1	GC-MS, RI$_1$, RI$_2$
Pinocarvone	–		0.4	GC-MS, RI$_1$, RI$_2$
Isobornyl acetate	–		0.5	GC-MS, RI$_1$, RI$_2$
Terpinen-4-ol	–		7.0	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
α-Terpineol	–		0.6	GC-MS, RI$_2$
α-Terpenyl acetate	–		0.1	GC-MS, RI$_1$, RI$_2$
Borneol	–		0.2	GC-MS, RI$_1$, RI$_2$
Neral	–		0.1	GC-MS, RI$_1$, RI$_2$
Nerol	–		tr‡	GC-MS, RI$_2$
Myrcen-8-ol	–		tr	GC-MS, RI$_2$
Benzyl isobutyrate	–		0.1	GC-MS, RI$_1$, RI$_2$
Sesquiterpene hydrocarbons	**62.6**		**28.1**	
δ-Elemene	0.6	GC-MS, RI$_1$, RI$_2$	0.9	GC-MS, RI$_1$, RI$_2$
α-Cubebene	–	GC-MS, RI$_1$, RI$_2$	0.1	GC-MS, RI$_1$, RI$_2$
α-Copaene	0.1	GC-MS, RI$_1$, RI$_2$	0.2	GC-MS, RI$_1$, RI$_2$
β-Bourbonene	5.6	GC-MS, RI$_1$, RI$_2$	0.2	GC-MS, RI$_1$, RI$_2$
β-Elemene	0.5	GC-MS, RI$_1$, RI$_2$	–	
β-Caryophyllene	6.1	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR	4.3	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
allo-Aromadendrene	0.7	GC-MS, RI$_1$, RI$_2$	–	
α-Humulene	2.7	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR	1.0	GC-MS, RI$_1$, RI$_2$
α-Gurjunene	1.4	GC-MS, RI$_1$, RI$_2$	–	
β-Gurjunene	0.7	GC-MS, RI$_1$, RI$_2$	–	
Germacrene-D	32.0	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR	1.9	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
Bicyclogermacrene	2.8	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR	5.6	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
δ-Cadinene	1.0	GC-MS, RI$_1$, RI$_2$	0.3	GC-MS, RI$_1$, RI$_2$
α-Bisabolene	–		10.3	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
(*E*,*E*)-α-Farnesene	8.0	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR	0.9	GC-MS, RI$_1$, RI$_2$
β-Bisabolene	–		0.6	GC-MS, RI$_1$, RI$_2$
Germacrene-B§	–		1.8	GC-MS, RI$_1$
α-Muurolene	0.4	GC-MS, RI$_1$, RI$_2$	–	
Oxygen-containing sesquiterpenes	**7.2**		**2.8**	
β-Caryophyllene epoxide	0.4	GC-MS, RI$_1$, RI$_2$	0.3	GC-MS, RI$_1$, RI$_2$
(6-*E*)-Nerolidol	1.4	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR	–	
Cubenol	–		0.2	GC-MS, RI$_1$, RI$_2$
Globulol	–		0.2	GC-MS, RI$_1$, RI$_2$
Spathulenol	1.3	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR	1.4	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR
T-Muurolol	–		0.4	GC-MS, RI$_1$, RI$_2$
α-Elemol	–		tr	GC-MS, RI$_2$
β-Bisabolol	–		0.2	GC-MS, RI$_1$, RI$_2$
α-Bisabolol	–		0.1	GC-MS, RI$_1$, RI$_2$
α-Cadinol	2.1	GC-MS, RI$_1$, RI$_2$, ^{13}C-NMR	–	
Furanodiene	tr	GC-MS, RI$_1$, RI$_2$	–	
1β,6α-dihydroxy-4(14)-eudesmene¶	2	^{13}C-NMR	–	

Table 2. Continued

Component	HC %	Identification method*	HB %	Identification method*
Others	–		**0.5**	
Hex-2-enal	–		0.2	GC-MS, RI_1, RI_2
Phytol	–		0.3	GC-MS, RI_1, RI_2
TOTAL	**74.3**		**83.7**	

*RI_1: Retention index in Supelcowax 10^{TM}. RI_2: Retention index in SE-30.

† Percentage less than 2%, not determined due to a superposition with an unidentified compound.

‡ tr: traces (⩽0.05%).

§ Tentatively identified.

¶ Estimated percentage obtained from the intensity of the signals of the ^{13}C-NMR analysis.

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