Essential oil of trees of the genus *Ocotea* (Lauraceae) in Costa Rica. I. *Ocotea brenesii*

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Abstract: The chemical composition of the essential oils from leaves and wood of *Ocotea brenesii* Standl. growing wild in Costa Rica was determined by capillary GC/FID and GC/MS. From the leaves, 64 compounds were identified, corresponding to 85.9% of the oil, and from the wood 57 compounds were identified corresponding to 69.0% of the oil. The major constituents identified in the leaf oil were α-copaene (21.1%), δ-cadinene (9.2%), spathulenol (7.3%), globulol (5.6%) and β-caryophyllene (5.2%). The major constituents of the wood oil were α-copaene (6.6%), caryophyllene oxide (6.3%), β-caryophyllene (6.1%) and humulene epoxide (4.6%). Rev. Biol. Trop. 53(3-4): 431-436. Epub 2005 Oct 3.

Key words: *Ocotea brenesii*, Lauraceae, essential oils composition, α-copaene, δ-cadinene, spathulenol, caryophyllene oxide, β-caryophyllene, globulol.

The Lauraceae is a family of about 2000-2200 species of mostly tropical trees. The family is an important component of cloud forests in Costa Rica where they occur in high abundance and diversity (Burger and van der Werff 1990, Haber 1996). This family is recognized by the simple, alternate, stiff and aromatic elliptic to obovate leaves, and by the fruits often borne in a cup.

Ocotea is a genus of 300-400 species growing mainly in the American tropics. The genus *Ocotea* belongs to the family Lauraceae and it is the largest genus of this family in Mesoamerica with 102 species (van der Werff 2002).

Ocotea brenesii is a tree of evergreen montane forest formations, from 700 to 2000 m elevation. The geographic distribution in Costa Rica ranges from near the "Alberto M. Brenes" Biological Preserve, San Ramón, to the area between Poás and Barva volcanoes in Alajuela Province (Burger and van der Werff 1990) and also is reported in Panama (van der Werff 2002). *O. brenesii* is recognized by the relatively broad leaves with a few secondary veins that appear thin in texture and dark in color when dry. Also it is characterized by racemose inflorescences and appressed indumenta on the young twigs (van der Werff 2002).

Chemically, the genus *Ocotea* is known mainly as a source of neolignans and benzylisoquinoline alkaloids (Gottlieb 1974, Franca *et al*. 1975, Ishige *et al*. 1991, Castro 1993, López *et al*. 1995). The chemical composition of the oils of some species belonging to this genus has been the subject of several studies (Mors 1959, Gottlieb and Magalhães 1960, Gottlieb *et al*. 1960a, 1960b, Brooks and Campbell 1969, Gottlieb *et al*. 1981, de Díaz *et al*. 1991, Terreaux et al. 1994, Reynolds and Kite 1995, Lorenzo *et al*. 2001, Bruni *et al*. 2004).

Previously, from the leaf of *Ocotea brenesii*, from Costa Rica, the isoquinoline alkaloids (+)-isocorydine, (-)-3-hydroxynuciferine and 3-hydroxy-6a,7-dehydronuciferine have been isolated (Lopez *et al*. 1996).

To the best of our knowledge no previous reports on the chemical composition of the oils of *O. brenesii* have been published.

MATERIALS AND METHODS

Fresh leaves and twigs of *Ocotea brenesii* Standl. [*Syn: Nectandra brenesii* (Standl.) C. K. Allen], Lauraceae, growing wild in Costa Rica were collected in April 2001, in San Ramón, Bajo Los Rodríguez, Province of Alajuela, Costa Rica. A voucher specimen was deposited at the Herbarium of the University of Costa Rica at the School of Biology (USJ 45808).

The leaves and dried wood were subject to hydrodistillation for 3 h using a modified Clevenger-type apparatus. The distilled oils were collected and dried over anhydrous sodium sulphate and stored in a freezer (0-10°C). The yield of the colorless oil from the leaf was 0.06% (v/w) and from the wood 0.02% (v/w).

The oils of *O. brenesii* were analyzed by GC/FID using a Shimadzu GC-17 gas chromatograph. The data were obtained on a 5% phenyl methylpolysiloxane fused silica capillary column (30 m x 0.25 mm; film thickness 0.20 µm), Heliflex (Alltech) AT-5, with a Shimadzu Class-VP, version 4.3 software. Operating conditions were: carrier gas N_2, flow 1.0 mL/min; oven temperature program: 60-220°C at 3°C/min, 220°C (10 min); sample injection port temperature 250°C; detector temperature 275°C; split 1:50.

The analysis by GC/MS was performed using a Shimadzu GC-17A gas chromatograph coupled with GCMS-QP5050 apparatus and CLASS 5000 software with Wiley138 computer database. The data were obtained on a 5% phenyl methylpolysiloxane fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 µm). Operating conditions were: carrier gas He, flow 1.0 mL/min; oven temperature program: 60-240°C at 3°C/min; sample injection port temperature 250°C; detector temperature 260°C; ionization voltage: 70eV; ionization current 60 µA; scanning speed 0.5 s over 38-400 amu range; split 1:70.

Identification of the components of the oils were performed using the retention indices on DB-5 type column, and by comparison of their mass spectra with those published in the literature (McLafferty 1993, Adams 1995, 2001) or those of our own database. Integration of the total chromatogram, expressed as area percent, has been used to obtain quantitative compositional data.

RESULTS

The chemical compositions of the leaf and wood oils of *O. brenesii* are presented in Tables 1 and 2. As it can be observed, 64 components were identified from leaves, representing *ca*. 86% of the oil. Sesquiterpenic hydrocarbons were the main constituents (53.4%) of the oil and contained α-copaene (21.1%), δ-cadinene (9.2%) and β-caryophyllene (5.2%) as the main compounds. The second group in quantity was constituted by oxygenated sesquiterpenes (29.9%); spathulenol (7.3%) and globulol (5.6%) are the main constituents.

From the wood, 57 compounds were identified, representing *ca*. 70% of the oil. The oxygenated sesquiterpenes (34.6%) and sesquiterpenic hydrocarbons (32.3%**)** are the principal constituents identified. The main constituents are α-copaene (6.6%), caryophyllene oxide (6.3%), β-caryophyllene (6.1%), globulol (5.0%) and humulene epoxide II (4.6%).

DISCUSSION

There are several reports on the composition of the oils of plants of the genus *Ocotea* from South America (Mors 1959, Gottlieb and Magalhães 1960, Gottlieb *et al*. 1960a, 1960b, Brooks and Campbell 1969, Gottlieb *et al*. 1981, de Díaz *et al*. 1991, Reynolds and Kite 1995, Lorenzo *et al*. 2001, Bruni *et al*. 2004) and Africa (Terreaux *et al*. 1994, Menut *et al*. 2002). The majority of them are characterized by the presence of phenylpropanoids like safrole and *O*-methyleugenol, cinnamaldehydes

TABLE 1
Percentage composition of the leaf oil of Ocotea brenesii

Compound[a]	RI[b]	%	Compound[a]	RI[b]	%
hexanal	807	t[c]	germacrene D	1481	3.2
Z-(E)-hexenal	855	0.4	β-selinene	1483	t
E-salvene	873	t	*trans*-muurola-4(14),5-diene	1490	t
2-heptanone	889	0.1	γ-amorphene	1494	0.9
heptanal	908	0.1	α-muurolene	1500	0.7
α-pinene	937	0.1	γ-cadinene	1512	0.2
5-methyl-3-heptanone	952	t	cubebol	1515	0.3
β-pinene	980	0.1	δ-cadinene	1527	9.2
1-octen-3-ol	980	t	*trans*-cadina-1(2),4-diene	1532	0.2
limonene	1031	t	α-cadinene	1537	0.1
1,8-cineole	1034	t	α-calacorene	1544	3.8
bergamal	1053	t	β-calacorene	1565	0.5
linalool	1099	0.1	*trans*-nerolidol	1568	0.2
nonanal	1106	0.1	spathulenol	1577	7.3
santalone	1181	t	globulol	1584	5.6
carvenone	1258	0.1	β-copaen-4-α-ol	1587	0.4
α-cubebene	1350	1.4	salvial-4(14)-en-1-one	1593	1.2
α-ylangene	1365	0.2	1-*epi*- cubenol	1627	2.1
isoledene	1366	0.5	caryophylla-4(14) ,8(15)-dien-5-α-ol	1638	0.3
α-copaene	1380	21.1	cubenol	1642	1.8
β-bourbonene	1384	0.2	α-muurolol	1647	1.3
β-cubebene	1390	2.0	α-cadinol	1653	0.2
β-elemene	1393	t	3-thujopsanone	1654	2.8
β-caryophyllene	1417	5.2	*trans*-calamenen-10-ol	1671	0.1
β-copaene	1428	0.5	cadalene	1674	1.0
γ-elemene	1435	0.1	eudesma-4(15),7-dien-1-β-ol	1686	3.0
α-*trans*-bergamotene	1436	t	10-nor-calamenen-10-one	1707	0.4
α-humulene	1451	0.6	cyclocolorenone	1760	0.5
allo-aromadendrene	1456	t	14-oxy-α-muurolene	1770	0.3
cis-muurola-4(14),5-diene	1455	t	14-hydroxy-α-muurolene	1778	0.2
trans-cadina-1(6),4-diene	1471	0.3	14-hydroxy-δ-cadinene	1798	0.2
γ-muurolene	1477	1.5	hexadecanoic acid	1968	1.6

Monoterpene hydrocarbons	0.2
Oxygenated monoterpenes	0.2
Sesquiterpene hydrocarbons	53.4
Oxygenated sesquiterpenes	29.9
Others	2.2

a Compounds listed in order of elution from 5% phenyl methyl silicone column.
b RI = Retention index relative to n-alkanes on the 5% phenyl methyl silicone column.
c t = Traces (<0.05%).

TABLE 2
Percentage composition of the wood oil of Ocotea brenesii

Compound[a]	RI[b]	%	Compound[a]	RI[b]	%
β-pinene	981	0.1	γ-amorphene	1495	1.6
linalool	1097	0.5	α-muurolene	1499	0.3
cis-*p*-menth-2-en-1-ol	1122	0.1	γ-cadinene	1513	0.1
α-campholenal	1127	0.1	cubebol	1516	2.9
trans-pinocarveol	1139	0.1	δ-cadinene	1525	3.8
trans-*p*-menth-2-en-1-ol	1146	0.1	*trans*-cadina-1(2),4-diene	1537	t
pinocarvone	1163	0.2	α-cadinene	1537	t
dill ether	1187	t[c]	α-calacorene	1543	1.7
α-terpineol	1191	t	*E*-nerolidol	1569	0.6
myrtenal	1198	0.3	caryophyllene oxide	1586	6.3
verbanol	1196	t	globulol	1587	5.0
trans-piperitol	1209	t	β-copaen-4-α-ol	1589	t
pulegone	1238	t	salvial-4(14)-en-1-one	1595	0.6
α-cubebene	1351	1.5	humulene epoxide II	1606	4.6
isoledene	1368	t	1-*epi*-cubenol	1629	1.5
α-copaene	1377	6.6	cubenol	1645	1.8
β-bourbonene	1384	0.1	α-cadinol	1650	t
β-cubebene	1391	2.2	3-thujopsanone	1654	2.3
β-elemene	1393	0.5	*cis*-calamenen-10-ol	1664	0.5
β-longipinene	1403	t	*trans*-calamenen-10-ol	1670	1.5
β-caryophyllene	1419	6.1	cadalene	1675	3.1
β-copaene	1427	0,1	eudesma-4(15),7-dien-1-β-ol	1688	0.3
α-*(E)*-ionone	1434	0.1	pentadecanal	1713	1.2
α-*trans*-bergamotene	1437	0.3	cyclocolorenone	1758	1.4
α-humulene	1452	0.9	14-oxy-α-muurolene	1765	3.2
γ-muurolene	1477	1.1	14-hydroxy-α-muurolene	1776	0.6
germacrene D	1489	1.6	14-hydroxy-δ-cadinene	1796	0.3
β-selinene	1484	0.4	hexadecanoic acid	1967	0.6
trans-muurola-4(14),5-diene	1489	0.2			

Monoterpene hydrocarbons	0.1
Oxygenated monoterpenes	1.4
Sesquiterpene hydrocarbons	32.3
Oxygenated sesquiterpenes	34.6
Others	0.6

a Compounds listed in order of elution from 5% phenyl methyl silicone column.
b RI = Retention index relative to n-alkanes on the 5% phenyl methyl silicone column.
c t = Traces (<0.05%).

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and some other benzenoid derivatives. The results obtained in this study showed that the oils of *O. brenesii* are characterized by compounds of terpenic chemical nature and are lacking of phenylpropanoids or benzenoid constituents that are typical of the majority of the *Ocotea* essential oils investigated previously.

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RESUMEN

Se determinó la composición química de los aceites esenciales obtenidos de las hojas y de la madera del árbol *Ocotea brenesii* Standl., perteneciente a la familia Lauraceae, que crece en forma silvestre en Costa Rica. Para el estudio se utilizaron técnicas de cromatografía de gases capilar con detector de ionización de flama (GC/FID) y cromatografía de gases capilar acoplada a un detector de espectrometría de masas (GC/MS). Del aceite volátil de las hojas fueron identificados 64 constituyentes, que correspondieron a un 85.9% del aceite. Del aceite de la madera se identificaron 57 compuestos que correspondieron a un 69.0% del aceite. Los constituyentes mayoritarios que se identificaron en el aceite de las hojas fueron α-copaeno (21.1%), δ-cadineno (9.2%), espatulenol (7.3%), globulol (5.6%) y β-cariofileno (5.2%). Los constituyentes mayoritarios del aceite de la madera fueron α-copaeno (6.6%), óxido de cariofileno (6.3%), β-cariofileno (6.1%) y el epóxido del humuleno (4.6%). La composición de los aceites es de naturaleza terpénica y no presentaron compuestos del tipo fenilpropanoide o bencenoide que son constituyentes característicos de la mayoría de los aceites de plantas del género *Ocotea* investigados anteriormente.

Palabras clave: Ocotea brenesii, Lauraceae, aceites esenciales, composición, α--copaeno, δ-cadineno, espatulenol, óxido de cariofileno, β-cariofileno, globulol.

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