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Essential oil of *Dittrichia viscosa* ssp. *viscosa*: analysis by ^{13}C-NMR and antimicrobial activity

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ABSTRACT: The essential oil of *Dittrichia viscosa* ssp. *viscosa* from Corsica was investigated by GC and ^{13}C-NMR spectroscopy. First, the sample was submitted to an acido-basic partition and the neutral part was repeatedly chromatographed. The analysis of all the fractions led to the identification of 71 components. The main constituents were fokienol (21.1%), (*E*)-nerolidol (8.6%) and eudesm-6-en-4α-ol (6.2%). The antimicrobial activity of the neutral and acidic fractions were investigated. While the neutral part appeared to be inactive, the acidic part was active against all the tested microorganisms. The highest activity was obtained against *Staphylococcus epidermidis*, *Streptococcus foecalis* and *Proteus vulgaris*. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: *Dittrichia viscosa* ssp. *viscosa*; essential oil composition; ^{13}C-NMR; antimicrobial activity

Introduction

Dittrichia viscosa (L.) W. Greuter is a perennial herbaceous plant of the family Compositae, growing wild in waste places of southern Europe. The extracts of the plant have been widely studied. Previous works led to the isolation and identification of eudesman-type acids,[1–3] sesquiterpene lactones,[2] nerolidol derivatives,[1,2] triterpenoids,[4] flavonoids,[5] fatty acids[4] and a diacylglycerol.[6]

D. viscosa is well known for a wide variety of biological activities. The extracts of the plant possessed antiviral and antioxidant properties.[7,8] Compounds isolated from the extracts exhibited antiinflammatory and gastric antiulcerous effects.[9,10] Moreover, a few studies reported on the antibacterial and antifungal properties of some compounds, such as sesquiterpene lactones or acids contained in the extracts of *D. viscosa*.[11–13]

The characterization of the essential oil of *Dittrichia viscosa* has been the subject of three works which revealed different compositions. An oil from Turkey contained borneol (25.2%), isobornyl acetate (22.5%) and bornyl acetate (19.5%) as major components.[14] A sample from Spain exhibited two acyclic sesquiterpene alcohols, fokienol (38.8%) and (*E*)-nerolidol (7.1%) as the main constituents.[15] Recently, an oil from Sardinia was reported to be dominated by globulol (16.8%), valerianol (12.0%) and caryophyllene oxide (8.0%).[16] Globulol (15.0%) and caryophyllene oxide (8.2%) were the major components of a supercritical CO_2 extract, accompanied by 8-isobutyryloxy isobornyl isobutyrate (13.1%) and viridiflorol (8.3%).[16]

In contrast with the extracts, another sample of the essential oil of *Dittrichia viscosa* from Sardinia appeared to be inactive against some microorganisms.[17] However, the composition of this oil was not reported.

The aim of the present work was to characterize the essential oil of *Dittrichia viscosa* ssp. *viscosa* from Corsica and to check its antimicrobial activity. The first investigations demonstrated that the oil is extremely complex, owing to the number of components and their unusual structures. So, in order to carry out a detailed analysis, we chose an approach based on the combination of repeated chromatographies and analysis of all the fractions by GC and ^{13}C-NMR. Concerning the antimicrobial properties, we evaluate independently the neutral and acidic fractions of *D. viscosa* essential oil.

Materials and methods

Plant material and oil production

The aerial parts of *Dittrichia viscosa* ssp. *viscosa* were collected at the full flowering stage in September 2001, near Ajaccio (Corsica, France). The essential oil was obtained by several hydrodistillations using a Clevenger-type apparatus for 4 h. The yields calculated from fresh material were 0.03–0.07%.

Acido-basic partition and chromatographic separation

The whole oil (14 g) was stirred (1 h) with 30 ml saturated solution of Na_2CO_3, then the mixture was extracted with diethyl oxide (3 × 20 ml). The organic layer, washed with

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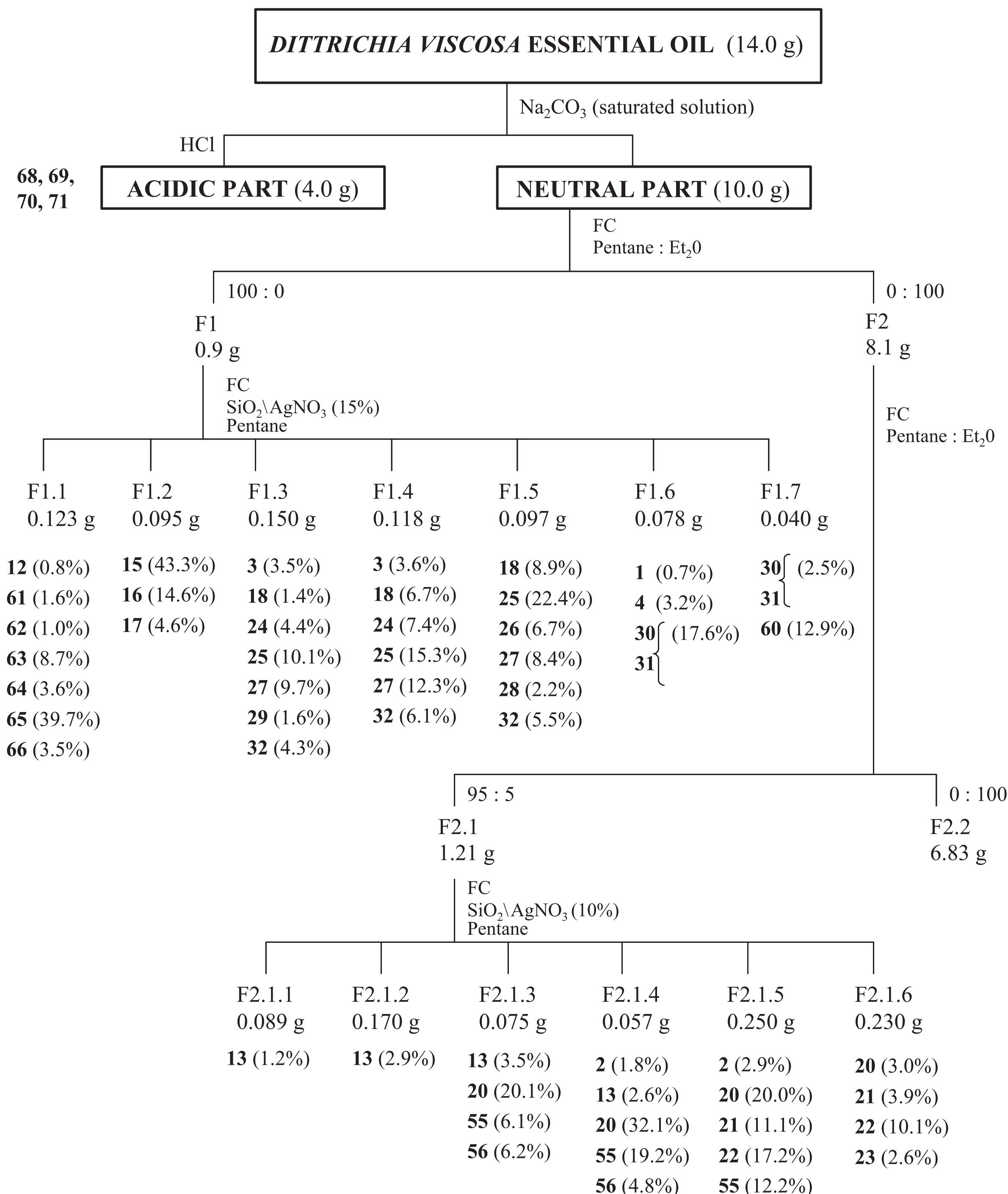
water until neutral pH, was dried over $MgSO_4$ and the solvent was removed under vacuum to yield 10.0 g of the neutral part. The aqueous layer was acidified with HCl (10%) and extracted with diethyl oxide (3 × 20 ml). The organic layer was washed with a saturated solution of $NaHCO_3$ to obtain neutral pH and dried over $MgSO_4$. Then the solvent was removed under vacuum to yield 4.0 g of the acidic part.

The neutral part (9 g) was chromatographed on a silica gel column (200–500 µm). Two fractions were eluted, F1 with pentane and F2 with diethyl oxide. Fraction F1 was further chromatographed on silica gel (35–70 µm) impregnated with $AgNO_3$ (15%) leading to seven fractions (F1.1–F1.7) by elution with pentane (Scheme 1). Fraction F2 was chromatographed on silica gel (35–70 µm) and two fractions (F2.1, F2.2) were eluted



Scheme 1. Fractionation of *Dittrichia viscosa* ssp. *viscosa* essential oil. The main components of each fraction and their percentages are reported by their number corresponding to the order of elution on apolar column (Table 1)

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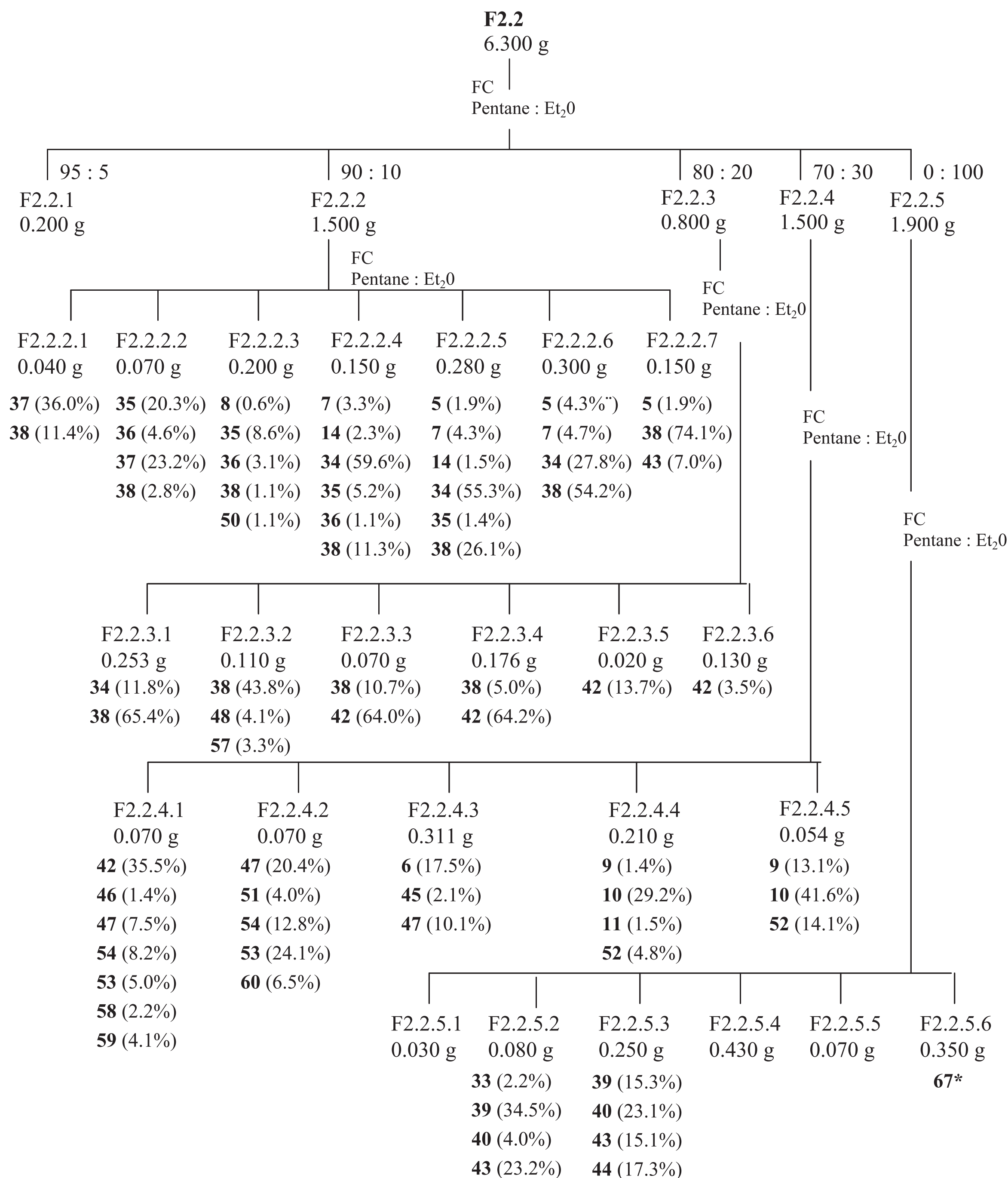
respectively with pentane:diethyl oxide (95:5) and diethyl oxide. Fraction F2.1 was chromatographed on silica gel (35–70 µm) impregnated with $AgNO_3$ (10%), giving six fractions (F2.1.1–F2.1.6) by elution with pentane:diethyl oxide (95:5) (Scheme 1). The fraction F2.2 was chromatographed on silica gel (35–70 µm) and five fractions (F2.2.1–F2.2.5) were eluted with mixtures of pentane/diethyl oxide of increasing polarity. Finally, fractions F2.2.2, F2.2.3, F2.2.4 and F2.2.5 were submitted to chromatography on silica gel (35–70 µm) with mixtures of pentane:diethyl oxide of increasing polarity on the basis of TLC analysis (Scheme 2).



Scheme 2. Separation of oxygenated fraction (F2.2). The main components of each fraction and their percentages are reported by their number corresponding to the order of elution on apolar column (Table 1). *Percentage not determined

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Analytical GC

GC analyses were carried out using a Perkin-Elmer Autosystem apparatus equipped with two flame ionization detectors (FID) and fused capillary columns (50 m × 0.22 mm i.d., film thickness 0.25 μm), BP-1 (polydimethylsiloxane) and BP-20 (polyethylenglycol). The oven temperature was programmed from 60 °C to 220 °C at 2 °C/min and then held isothermal (20 min). Injector temperature was 250 °C (injection mode, split). The relative proportions of constituents in the neutral part and in the subfractions of chromatography were expressed as percentages obtained by area normalization, without using correcting factors. Retention indices (RI) were determined relative to the retention times of a series of *n*-alkanes with linear interpolation (Target Compounds software from Perkin-Elmer).

^{13}C-NMR analysis

All NMR spectra were recorded on a Bruker AC 200 Fourier Transform Spectrometer, operating at 50.323 MHz, equipped with a 10 mm probe (200 mg oil, 2 ml $CDCl_3$, 5000 scans) or a 5 mm probe (70 mg oil, 0.5 ml $CDCl_3$, 10 000 scans) in deuterated chloroform, with all shifts referred to internal tetramethylsilane (TMS). ^{13}C-NMR spectra were recorded with the following parameters: pulse width (PW), 5 μs (or 3 μs) (flip angle 45°); acquisition time, 1.3 s for 32 k data table with a spectral width (SW) of 12 500 Hz (250 ppm); CPD mode decoupling; digital resolution, 0.763 Hz/pt. Exponential line broadening multiplication (LB = 1 Hz) of the free induction decay (FID) was applied before Fourier transformation.

Component identification

Neutral part and all the fractions of chromatography were analysed by ^{13}C-NMR spectroscopy and GC while acidic part was only analysed by ^{13}C-NMR following a methodology developed and computerized in our laboratory.[18] The computer program compared the chemical shift of each carbon of the compounds in the experimental spectrum with the spectra of pure components listed in our data bank. The identification of all the compounds was carried out, taking into account: (a) the number of observed carbons with respect to the number of expected signals; (b) the number of overlapped signals of carbons which possess fortuitously the same chemical shift; (c) the difference of the chemical shift of the signals in the mixture spectrum with those of reference spectra compiled in the laboratory spectral library.

The occurrence of the individual components was ensured by comparison of the GC retention indices on apolar and polar columns with those of authentic samples or literature data.

Antimicrobial activity

Antibacterial and antifungal activities of the neutral and acidic parts, obtained after acido-basic treatment, were evaluated against three Gram-positive (*Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *Streptococcus faecalis* CECT 795) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Proteus vulgaris* CECT 484), two yeasts (*Candida albicans* CECT 1394 and *Cryptococcus neoformans* CECT 1078) and three filamentous fungi (*Cladosporium cladosporioides* CECT 2111, *Aspergillus niger* CECT 2574 and *A. fumigatus* CECT 2071). Minimal inhibitory concentration (MIC) was evaluated by an agar dilution technique modified and adapted to multiwell microtitre plates (96 wells), using Mueller–Hinton agar for bacteria and Sabouraud dextrose for yeasts and filamentous fungi.[19] Different concentrations of the essential oils were obtained in dimethyl sulphoxide (DMSO) to give serial two-fold dilutions that were added to each well, resulting in concentrations of 0.156–20 μl/ml. The final concentration of DMSO never exceeded 2%. The wells were inoculated with test microorganism suspensions at a final inoculum of 10^4 cells/ml for bacteria and yeast and 10^4–10^5 spores/ml for filamentous fungi.

For each strain tested, the growth conditions and the sterility of the medium were checked in two control columns. The inoquity of the DMSO were also checked at the highest tested concentration. The microtitre plates were then incubated for 24 h at 37 °C for the bacteria and for 48 h at 27 °C for yeast and filamentous fungi. Standard antibiotics (chloramphenicol and nystatin) were used in order to control the sensitivity of the tested organisms.

Results and discussion

The chromatographic profile and ^{13}C-NMR spectrum of *Dittrichia viscosa* essential oil suggested the occurrence of a large number of oxygenated sesquiterpenes as well as acidic compounds. So, the essential oil was first submitted to an acido-basic treatment and the neutral and acidic parts were analysed separately. The neutral part was submitted to repeated chromatographies and all the fractions were analysed by ^{13}C-NMR with the help of a laboratory-produced software (see Materials and methods). The chemical composition of *Dittrichia viscosa* essential oil is presented in Table 1.

Analysis of the neutral part and all the fractions of chromatography led to the identification of 67 constituents, among which 43 were oxygenated and 24 hydrocarbon compounds, including seven linear alcanes.

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Table 1. Chemical composition of the essential oil of *Dittrichia viscosa* ssp. *viscosa*

No.	Compounds	(%)[#]	RI BP-1	RI BP-20	Fraction	Ref.
	Neutral part					
1	*p*-Cymene	0.5	1012	1274	F1	
2	Cineole	1.7*	1021	1212	NP	
3	Limonene	0.4*	1021	1204	F1	
4	*p*-Cymenene	0.2	1072	1140	F1	
5	Linalool	1.0	1082	1546	NP	
6	*p*-Cymen-8-ol	0.6	1159	1852	F2.2.4.2	
7	Terpinen-4-ol	} 1.0	1161	1601	NP	
8	Neomenthol		1161	1601	F2.2.2.3	
9	*p*-Mentha-1(7),2-dien-8-ol	0.9	1168	1783	F2.2.4.4	
10	α-Terpineol	0.8	1172	1695	NP	
11	Nerol	0.1	1208	1800	F2.2.4.4	
12	Tridecane	tr	1300	1300	F1.1	
13	α-Terpinyl acetate	0.3	1332	1695	F2.1.1	
14	Eugenol methyl	0.4	1367	2017	F2.2.2.2	
15	α-Ylangene	0.4	1372	1483	F1	
16	α-Copaene	0.2	1381	1483	F1.2	
17	Sativene	0.1	1391	1526	F1.2	
18	(*E*)-β-Caryophyllene	0.7	1419	1597	F1	
19	Geranyl acetone	0.1	1427	1857	F2.2.1	
20	3,6;6,9-Bisepoxyfarnesa-1,7(14),10-triene	1.7*	1450	1831	F2.1.3	23
21	9-*epi*-3,6;6,9-Bisepoxyfarnesa-1,7(14),10-triene	0.9*	1450	1834	F2.1.5	23
22	3-*epi*-3,6;6,9-Bisepoxyfarnesa-1,7(14),10-triene	1.4*	1458	1870	F2.1.5	23
23	3,9-*diepi*-3,6;6,9-Bisepoxyfarnesa-1,7(14),10-triene	0.3*	1458	1825	F2.1.6	23
24	γ-Muurolene	0.2	1466	1688	F1.3	
25	α-Amorphene	1.1	1475	1688	F1	
26	β-Selinene	0.2	1488	1715	F1	
27	α-Muurolene	0.6	1493	1724	F1	
28	α-Selinene	0.1	1500	1719	F1	
29	γ-Cadinene	0.2	1507	1760	F1.3	
30	*trans*-Calamenene	} 0.2	1510	1835	F1.6	
31	*cis*-Calamenene		1510	1835	F1.6	25
32	δ-Cadinene	0.2	1515	1758	F1	
33	7,10-Epoxyfarnesa-1,5,11-trien-3-ol	0.1	1527	2122	F2.2.5.2	23
34	(*E*)-Nerolidol	8.6	1547	2043	NP	
35	*trans*-Bejarol	0.6	1557	2051	F2.2.2.2	26
36	*cis*-Bejarol	0.4	1559	2060	F2.2.2.2	26
37	Caryophyllene oxide	2.5*	1576	1987	NP	
38	Fokienol	21.1♣	1576	2170	NP	
39	3-*epi*-6,9-Epoxyfarnesa-1,7(14),10-trien-3-ol	1.9*	1601	2255	F2.2.5.2	23
40	6,9-Epoxyfarnesa-1,7(14),10-trien-3-ol	1.8*	1601	2257	F2.2.5.2	23
41	Humulene oxide II	0.3*	1601	2044	F2.2.1	
42	Eudesm-6-en-4α-ol	6.2	1607	2170	F2	27
43	6-*epi*-6,9-Epoxyfarnesa-1,7(14),10-trien-3-ol	1.3*	1614	2276	F2.2.5.2	23
44	3,6-diepi-6,9-Epoxyfarnesa-1,7(14),10-trien-3-ol	0.9*	1614	2284	F2.2.5.3	23
45	γ-Eudesmol	0.1*	1620	2172	F2.2.4.2	
46	Alismol	0.3*	1620	2295	F2.2.4.1	
47	Caryophylla-4(14),8(15)-dien-5α-ol	1.9	1623	2302	NP	
48	τ-Muurolol	0.1*	1629	2188	F2.2.3.2	
49	τ-Cadinol	0.1*	1629	2172	F2.2.3.2	
50	1-epi-Cubenol	0.1	1630	2067	F2.2.2.3	
51	β-Eudesmol	} 1.0	1641	2234	F2.2.4.3	
52	α-Cadinol		1641	2234	F2.2.4.5	
53	Selin-11-en-4α-ol	1.6*	1641	2256	F2	
54	α-Eudesmol	2.2	1643	2224	NP	
55	Porosadien-7-one	1.5	1656	2160	F2.1.4	24
56	Benzyl benzoate	0.4	1725	2618	F2.1.3	
57	β-Cyperone	0.2	1766	2403	F2.2.3.2	28
58	3-Hydroxy-3,7,11-trimethyldodeca-1,6(*E*),10-trien-9-yl propanoate	0.3	1757	2378	F2.2.4.1	2
59	3-Hydroxy-3,7,11-trimethyldodeca-1,6(*E*),10-trien-9-yl isobutyrate	0.6	1881	2475	F2.2.4.2	2
60	Cadalene	0.2*	1655	2231	F1.7	
61	Heneicosane	0.4	2100	2100	F1.1	
62	Docosane	0.1*		2200	F1.1	
63	Tricosane	0.5*		2300	F1.1	
64	Tetracosane	0.4*		2400	F1.1	
65	Pentacosane	0.3*		2500	F1.1	
66	Hexacosane	0.1*		2600	F1.1	
67	3,7,11-Trimethyldodeca-1,6(*E*),9-trien-3,11-diol (Triendiol)	—	£	£	F2.2.5.6	1
	Monoterpene hydrocarbons	1.1				

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Table 1. (*Continued*)

No.	Compounds	(%)#	RI BP-1	RI BP-20	Fraction	Ref.
	Oxygenated monoterpenes	6.5				
	Sesquiterpene hydrocarbons	4.4				
	Oxygenated sesquiterpenes	60.0				
	Others	2.7				
	Acidic part[20]					
68	Eudesma-3,11(13)-dien-12-oic acid	43.7	£	£		20
69	Eudesma-4,11(13)-dien-12-oic acid	10.9	£	£		21
70	Costic acid	4.8	£	£		1
71	Eudesma-2,4(15),11(13)-trien-12-oic acid	1.9	£	£		1

Order of elution and percentage are given as measured on apolar column (BP-1), except for compounds marked *, percentage on BP-20. RI: retention indices measured respectively on apolar and polar columns.
♣ The ratio of fokienol on apolar column has been evaluated by subtraction of the percentage of caryophylene oxide on polar column.
tr, <0.05%. NP, neutral part. F1, F1.1, fractions where the compound was identified for the first time. £, retention indices not determined.

The neutral part of *Dittrichia viscosa* essential oil was characterized by a high content of sesquiterpenes (64.4%), mostly oxygenated ones (60.0%), with fokienol (21.1%), (*E*)-nerolidol (8.6%) and eudesm-6-en-4α-ol (6.2%) as the main components. Monoterpenes and linear alcanes were present at much smaller amounts, 7.6% and 1.8% respectively.

Among the identified sesquiterpenes, 28 were determined by comparison of their ^{13}C-NMR spectral data with those of authentic samples compiled in our laboratory-made library. Eighteen components which represented 20.0% of the total amount of the neutral part, were identified by computer-aided searching of ^{13}C-NMR data available in the literature (Table 1). Linear alcanes were identified by GC–RI, the characteristic chemical shifts of the signals being observed in the ^{13}C-NMR spectra of the corresponding fractions (Table 1).

Analysis of the acidic part by ^{13}C-NMR led to the identification of four eudesman-type acids[20] (Figure 1): eudesma-3,11(13)-dien-12-oic acid (isocostic acid),[21] costic acid (identified by comparison of NMR data with those of an authentic sample) and eudesma-2,4(15),11(13)-trien-12-oic acid,[1] (2,3-didehydrocostic acid) already reported in the extracts of *Dittrichia viscosa*, and eudesma-4,11(13)-dien-12-oic acid[22], reported for the first time in *Dittrichia* genus. Instead of the conventional two-step sequence (derivatization-GC analysis) we developed and applied a quantitative procedure based on ^{13}C-NMR spectroscopy, using an internal standard.[20] The four sesquiterpene acids represented 43.7%, 15.8%, 1.9% and 10.9%, respectively, of the acidic part.

The sesquiterpenes identified in *Dittrichia viscosa* essential oil exhibited diverse carbon skeletons: farnesane, bicyclo[4.4.0]decane (eudesmane, cadinane, muurolane), caryophyllane and polycyclic. Among the farnesane derivatives (Figure 2), we can notice the occurrence of nine unusual sesquiterpenes: four bisepoxyfarnesatrienes (**20**–**23**) and five epoxyfarnesatrienols (**33**, **39**, **40**, **43** and **44**) first reported in *Tanacetum fruticulosum*.[23] Usually, the identification of stereoisomeres by conventional

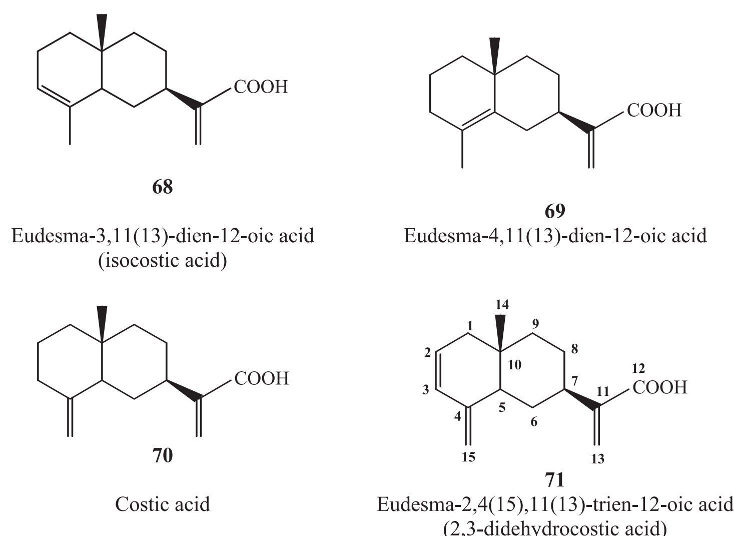

Figure 1. Structures of eudesman-type acids of *Dittrichia viscosa* ssp. *viscosa* essential oil

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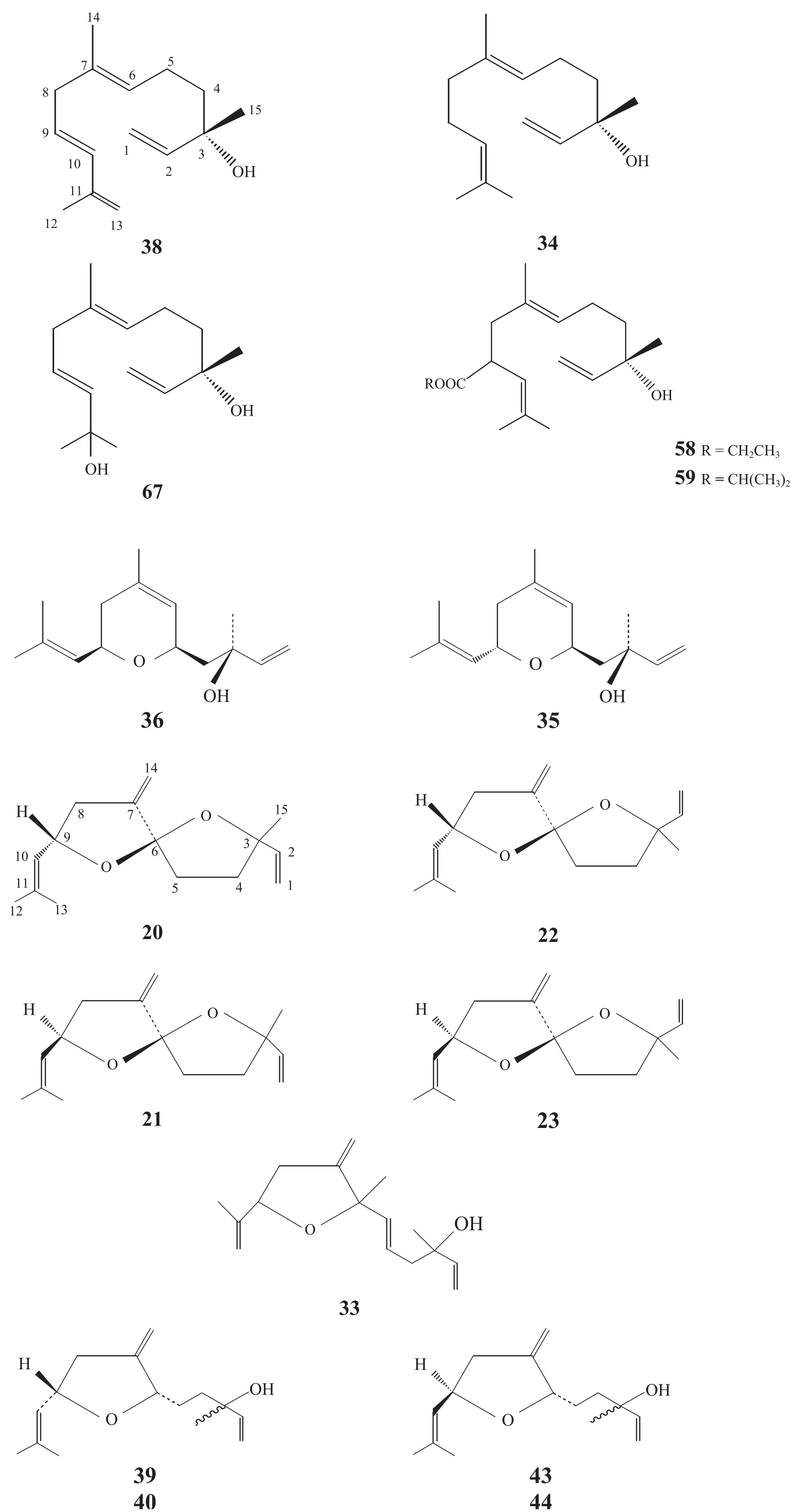

Figure 2. Structures of farnesane derivatives of *Dittrichia viscosa* ssp. *viscosa* essential oil

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Table 2. Minimal inhibitory concentration (MIC) of *Dittrichia viscosa* ssp. *viscosa* essential oil

Test organism	MIC			
	Essential oil (μl/ml)		Controls (μg/ml)	
	Neutral part	Acidic part	Chloramphenicol	Nystatin
Escherichia coli	—[a]	—	12.5	NT[b]
Staphylococcus aureus	—	2.5	12.5	NT
Staphylococcus epidermidis	—	1.25	12.5	NT
Streptococcus faecalis	—	1.25	12.5	NT
Proteus vulgaris	—	1.25	6.25	NT
Candida albicans	—	10.0	NT	3.13
Cryptococcus neoformans	—	2.5	NT	3.13
Cladosporium cladosporioides	—	2.5	NT	12.5
Aspergillus niger	—	10.0	NT	6.25
Aspergillus fumigatus	—	10.0	NT	12.5

[a] No activity.
[b] Not tested.

techniques is quite difficult and requires the purification of the components. Conversely, using ^{13}C-NMR spectroscopy, even a very slight structural modification induces measurable chemical shift variations at most, if not all, carbons of the molecule. So, despite the similarity of the molecules, four bisepoxyfarnesatrienes on the one hand and five epoxyfarnesatrienols on the other hand, have been identified simultaneously in several fractions. The occurrence of the porosadien-7-one [6(7 $\rightarrow$ 8)-*abeo*-eudesmadienone], first isolated in *Phoebe porosa* essential oil and which exhibited the bicyclo[4.3.0]nonane framework,[24] should be noted.

The composition of *Dittrichia viscosa* ssp. *viscosa* essential oil from Corsica showed some similarities with the one from Spain, particularly concerning the contents of fokienol and (*E*)-nerolidol.[15] However, in their short report the authors did not mention the occurrence of several sesquiterpenes and particularly the eudesman-type acids as well as the farnesane derivatives. Conversely, the compositions of the Turkish oil, exhibiting bornyl acetate, isobornyl acetate and borneol as main components,[14] and that of the Sardinian oil, dominated by globulol, valerianol and caryophyllene oxide,[16] differed drastically from that of our sample.

Concerning the antimicrobial activity of *Dittrichia viscosa* essential oil, the acidic and neutral parts have been evaluated separately (Table 2). The neutral part appeared to be inactive against all the tested organisms, while the acidic part was active against all the microorganisms, except *Escherichia coli*. The latter exhibited 2.5 μl/ml MIC values for *Staphylococcus aureus*, *Cryptococcus neoformans* and *Cladosoporium cladospotioides* and displayed the highest activity against *Staphylococcus epidermidis*, *Streptococcus foecalis* and *Proteus vulgaris* (MIC value, 1.25 μl/ml). In contrast, *Dittrichia viscosa* Sardinian essential oil was inactive against these microorganisms.[17]

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References

1. Grande M, Bellido IS. *J. Nat. Prod.* 1992; **55**: 1074–1083.
2. Sanz JF, Ferrando C, Marco JA. *Phytochemistry* 1991; **30**: 3653–3655.
3. Ulubelen A, Oksüz S, Gören N. *Phytochemistry* 1987; **26**: 1223–1224.
4. Grande M, Torres P, Piera F, Bellido IS. *Phytochemistry* 1992; **31**: 1826–1828.
5. Grande M, Piera F, Cuenca A, Torres P, Bellido IS. *Planta Med.* 1985; **51**: 414–419.
6. Mañez S, Carmen Recio M, Gil I *et al. J. Nat. Prod.* 1999; **62**: 601–604.
7. Abad MJ, Guerra JA, Bermejo P, Irurzun A, Carrasco L. *Phytother. Res.* 2000; **14**: 604–607.
8. Schinella GR, Tournier HA, Prieto JM, Mordujovich P, Rios JL. *Life Sci.* 2002; **70**: 1023–1033.
9. Hernandez V, Carmen Recio M, Mañez S *et al. Planta Med.* 2001; **67**: 726–731.
10. Alarcon de La Lastra C, Lopez A, Moltiva V. *Planta Med.* 1993; **59**: 497–501.
11. Maoz M, Kashman Y, Neeman I. *Planta Med.* 1999; **65**: 281–283.
12. Abou-Jawdah Y, Sobh H, Salama A. *J. Agric. Food Chem.* 2002; **50**: 3208–3213.
13. Shtacher G, Kashman Y. *J. Med. Chem.* 1970; **13**: 1221–1223.
14. Perez-Alonzo MJ, Velasco-Negueruela A, Emin Duru M, Harmandar M, Garcia Vallejo MC. *Flavour Fragr. J.* 1996; **11**: 349–351.
15. Camacho A, Fernandez A, Fernandez C, Altarejos J, Laurent R. *Riv. Ital. EPPOS* 2000; **29**: 3–8.
16. Marongiu B, Piras A, Pani F, Porcedda S, Ballero M. *Flavour Fragr. J.* 2003; **18**: 505–509.
17. Bonsignore L, Loy G, Secci D, De Logu A, Palmieri G. *Fitoterapia* 1990; **LXI**: 339–341.
18. Tomi F, Bradesi P, Bighelli A, Casanova J. *J. Magn. Reson. Anal.* 1995; **1**: 25–34.
19. Salgueiro LR, Cavaleiro C, Gonçalves MJ, Proença da Cunha A. *Planta Med.* 2003; **69**: 83–86.
20. Blanc M-C, Bradesi P, Casanova J. *Phytochem. Anal.* 2005; **16**: 150–154.

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21. Lanzetta R, Lama G, Mauriello G *et al*. *Phytochemistry* 1991; **30**: 1121–1124.
22. Ceccherelli P, Curini M, Marcotullio MC, Rosati O. *Tetrahedr*. *Lett*. 1990; **21**: 3071–3074.
23. Weyerstahl P, Marshall H, Thefeld K, Rustaiyan A. *Flavour Fragr*. *J*. 1999; **14**: 112–120.
24. Weyerstahl P, Marshall H, Splittgerber U. *Liebigs Ann. Chem.* 1994; **5**: 523–525.
25. Katsuyuki N, Masashi I, Masakazu S *et al*. *Molecules* 2002; **7**: 517–527.
26. Pascual Teresa J, Bellido IS, Gonzalez MS, Vicente S. *Phytochemistry* 1984; **23**: 2064–2065.
27. Blay G, Schrijvers R, Winjnbers J, De Groot A. *J*. *Org*. *Chem*. 1995; **60**: 2188–2194.
28. Blay G, Cardona L, Garcia B, Pedro JR. *Tetrahedron* 1996; **52**: 10507–10518.

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