Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 1956–1962
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

β-Cyclolavandulyl and β-isocyclolavandulyl esters from *Peucedanum paniculatum* L., an endemic species to Corsica

Muriel Vellutini, Nicolas Baldovini [1], Dominique de Rocca Serra, Félix Tomi, Joseph Casanova *

Université de Corse, UMR-CNRS 6134, Equipe Chimie et Biomasse, Route des Sanguinaires, 20 000 Ajaccio, France

Received 18 March 2005; received in revised form 1 July 2005; accepted 1 July 2005
Available online 15 August 2005

Abstract

Eight cyclolavandulyl esters (β-cyclolavandulyl and β-isocyclolavandulyl acetate, propionate, isobutyrate, isovalerate) were identified in the leaf and root oils of *Peucedanum paniculatum* L., an endemic species to Corsica, by comparison of their spectroscopic data with those of synthetic material. Their structures were elucidated by spectroscopic methods. The antimicrobial activity of essential oils of leaves and roots was evaluated against eleven microorganisms.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: *Peucedanum paniculatum* L.; Apiaceae; β-Cyclolavandulyl esters; β-Isocyclolavandulyl esters; 2D NMR; Antimicrobial activity

1. Introduction

Peucedanum paniculatum L., syn. *Peucedanum officinale* subsp. *paniculatum* (Loisel) Frey. is an Apiaceae endemic to Corsica (Gamisans and Jeanmonod, 1993). This perennial plant, 70–120 cm high, grows wild above 600 m in the centre and northwest of the island (Gamisans and Marzocchi, 1996). It thrives especially in the periodically burnt pasture land, its deep tap root makes it well adapted to withstand fire. This species, morphologically close to fennel, is known for its important phototoxicity and is rejected by cattle. This plant has also a determinant impact on the growth and distribution of *Papilio hospiton* Géné, an endemic Corsican butterfly (Aubert et al., 1996).

Continuing our research on essential oil bearing plants growing wild in Corsica, we report on the chemical composition and antimicrobial activity of the essential oils obtained from leaves and roots of *P. paniculatum* L. Combination of chromatographic partition, hemisynthesis and structural analysis led to the identification of eight new natural irregular monoterpene esters.

2. Results and discussion

Essential oils of *P. paniculatum* were obtained by separate hydrodistillation of the roots and the leaves. Both oils were submitted to analysis by GC, GC–MS and ^{13}C NMR following the pioneering work of Formácek and Kubeczka (1982) and a methodology developed and computerised in our laboratories (Tomi et al., 1995; Rezzi et al., 2002). Although this procedure allowed the identification of a few minor compounds, all the major components remained unidentified. In order to characterise both oils, we adopted a different strategy

* Corresponding author. Tel.: +33 4 95 52 41 21; fax: +33 4 95 52 41 42.
E-mail address: joseph.casanova@univ-corse.fr (J. Casanova).
[1] Present address: Université de Nice-Sophia Antipolis, Faculté des Sciences, Laboratoire Arômes, Synthèses et Interactions, Parc Valrose, 06108 Nice Cedex 2, France.

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2005.07.001

Table 1
Chemical composition of leaf and root oils from *Peucedanum paniculatum* L.

Components	RI_a	RI_p	Leaf oil	Root oil	Identification mode
Sabinene	962	1115	0.8	0.5	RI, MS, ^{13}C NMR
Myrcene	981	1161	0.1	–	RI, MS, ^{13}C NMR
Limonene	1024	1201	3.8	0.5	RI, MS, ^{13}C NMR
(*Z*)-β-Ocimene	1025	1233	2.2	–	RI, MS, ^{13}C NMR
(*E*)-β-Ocimene	1036	1250	1.0	–	RI, MS, ^{13}C NMR
Terpinolene	1079	1281	0.5	0.1	RI, MS, ^{13}C NMR
Lavandulol	1149	1673	0.5	1.3	RI, MS, ^{13}C NMR
Terpineol-4	1160	1602	0.2	–	RI, ^{13}C NMR
β-Isocyclolavandulol	1163	1759	2.2	5.7	RI, MS, ^{13}C NMR
β-Cyclolavandulol	1184	1744	4.3	5.0	RI, MS, ^{13}C NMR
β-Cyclolavandulal	1207	1656	3.0	0.5	RI, MS, ^{13}C NMR
Decanol	1254	1764	0.3	–	RI, ^{13}C NMR
Lavandulyl acetate	1273	1607	3.0	9.8	RI, MS, ^{13}C NMR
β-Isocyclolavandulyl acetate	1283	1634	6.1	15.8	RI, MS, ^{13}C NMR
β-Cyclolavandulyl acetate	1303	1655	16.1	13.9	RI, MS, ^{13}C NMR
β-Isocyclolavandulyl propionate	1374	1694	1.3	3.7	RI, MS, ^{13}C NMR
α-Copaene	1377	1487	0.3	0.1	RI, ^{13}C NMR
β-Bourbonene	1385	1516	1.1	–	RI, MS, ^{13}C NMR
β-Elemene	1388	1589	4.0	–	RI, MS, ^{13}C NMR
β-Cyclolavandulyl propionate	1391	1712	2.0	4.6	RI, MS, ^{13}C NMR
Lavandulyl isobutyrate	1405	1674	1.2	0.4	RI, ^{13}C NMR
β-Isocyclolavandulyl isobutyrate	1418	1691	5.3	7.3	RI, MS, ^{13}C NMR
(*E*)-Caryophyllene	1420	1593	0.8	3.2	RI, ^{13}C NMR
β-Cyclolavandulyl isobutyrate	1432	1702	17.8	6.2	RI, MS, ^{13}C NMR
α-Humulene	1453	1667	–	0.2	RI, MS, ^{13}C NMR
γ-Muurolene	1471	1681	0.6	–	RI, MS, ^{13}C NMR
Germacrene-D	1478	1704	2.5	–	RI, MS, ^{13}C NMR
β-Selinene	1483	1716	0.4	0.1	RI, MS, ^{13}C NMR
Bicyclogermacrene	1494	1727	0.4	–	RI, MS, ^{13}C NMR
α-Selinene	1495	1720	0.3	0.2	RI, MS, ^{13}C NMR
δ-Cadinene	1515	1755	0.5	0.1	RI, MS, ^{13}C NMR
β-Isocyclolavandulyl isovalerate	1520	1788	2.2	1.5	RI, MS, ^{13}C NMR
β-Cyclolavandulyl isovalerate	1525	1815	0.3	0.2	RI, MS, ^{13}C NMR
Selina-3,7(11)-diene	1540	–	0.1	–	RI, ^{13}C NMR
Spathulenol	1565	2124	0.7	–	RI, MS, ^{13}C NMR
Caryophyllene oxide	1573	1983	0.4	0.4	RI, MS, ^{13}C NMR
Globulol	1576	2076	0.1	–	RI, MS, ^{13}C NMR
neo-Intermedeol[a]	1601	2139	0.1	–	RI, ^{13}C NMR
epi-Cubenol	1617	2066	0.1	–	RI, ^{13}C NMR
τ-Muurolol	1636	2171	0.1	–	RI, MS, ^{13}C NMR
Selin-11-en-4α-ol	1639	2253	0.5	–	RI, MS, ^{13}C NMR
α-Cadinol	1643	2231	0.1	–	RI, ^{13}C NMR
Tetradecanol	1653	2168	0.1	–	RI, ^{13}C NMR
4,5-*epi*-Cryptomeridiol[a]	–	–	X	–	^{13}C NMR
4(15)-Eudesmene-1β,6α-diol[a]	–	–	X	–	^{13}C NMR
Total			87.4	81.3	

Order of elution and percentages of individual components are given on BP-1 column: RI_a, retention index on BP-1 apolar column; RI_p, retention index on BP-20 polar column.

[a] *neo*-Intermedeol, 4,5-*epi*-cryptomeridiol, 4(15)-eudesmene-1β,6α-diol were identified by comparison with literature data (Kesselmans and Wijnberg, 1992; Ahmad et al., 1992; Su et al., 1995).

for the analysis of leaf and root oils. Both chemical compositions are reported in Table 1.

2.1. Leaf oil

Analysis of the leaf oil by GC, GC–MS and ^{13}C NMR, and the fractions obtained after repeated chromatography by GC and ^{13}C NMR, led to the identification of several monoterpenes and sesquiterpenes including two irregular monoterpenes, β-cyclolavandulal **1** and β-cyclolavandulol **2**. However, most of the oxygenated components, including the major ones, remained unidentified. Examination of the unassigned signals in the NMR spectra of the chromatographic fractions led us to suspect that unidentified components could be β-cyclolavandulyl esters and more precisely acetate **3**,

Table 2
^{1}H NMR spectral data of β-cyclolavandulol **2** and β-cyclolavandulyl esters **3–6**

H	2	3	4	5	6
2	2.13; *m*	2.08; *m*	2.05; *m*	2.05; *m*	2.06; *m*
3	1.36; *t* (6.5)	1.36; *t* (6.5)	1.38; *t* (7.0)	1.35; *t* (6.6)	1.35; *t* (6.5)
5	1.75; *s*	1.77; *s*	1.77; *s*	1.77; *s*	1.76; *s*
7	4.12; *s*	4.58; *s*	4.59; *s*	4.59; *s*	4.59; *s*
8	1.68; *s*	1.68; *s*	1.68; *s*	1.68; *s*	1.68; *s*
9/10	0.89; *s*	0.89; *s*	0.88; *s*	0.88; s	0.88; *s*
12		2.05; *s*	2.35; *q* (7.5)	2.55; *h* (7.0)	2.18; *m*
13			1.14; *t* (7.5)		2.10; *m*
13/14				1.15; *d* (7.0)	
14/15					0.94; *d* (7.2)

Table 3
^{13}C NMR spectral data of β-cyclolavandulol **2** and β-cyclolavandulyl esters **3–6**

C	2	3	4	5	6
1	128.00	123.68	123.73	123.91	123.73
2	25.44	25.61	25.55	25.54	25.64
3	35.66	35.51	35.47	35.52	35.46
4	29.03	29.02	29.01	29.03	29.00
5	45.95	46.07	46.03	46.07	45.99
6	129.99	132.32	132.17	131.96	132.20
7	62.88	64.62	64.48	64.43	64.29
8	19.03	19.27	19.27	19.28	19.27
9	28.20	28.18	28.17	28.15	28.14
10	28.20	28.18	28.17	28.15	28.14
11		171.32	174.79	177.34	173.39
12		20.98	27.63	34.13	43.52
13			9.23		25.77
13/14				19.07	
14/15					22.39

propionate **4**, isobutyrate **5** and isovalerate **6**. In our hands, the suspected β-cyclolavandulyl esters proved to be difficult to isolate by the usual column chromatographic procedure. So, we prepared the esters **3–6** from β-cyclolavandulol **2**, itself synthesised from lavandulol according to the procedure of Kuhn and Schinz (1952). The NMR data are reported in Tables 2 and 3. Then, comparison of the spectral data of the reference compounds with those of the fractions of chromatography allowed the identification of the esters **3–6** in the leaf oil of *P. paniculatum*. Subsequently, we observed that some minor components, which remained unidentified in the leaf oil, were present at higher content in the root oil. It is also noteworthy that the analysis of the chromatographic fractions by ^{13}C NMR allowed the identification of two sesquiterpene diols, 4,5-*epi*-cryptomeridiol and 4(15)-eudesmen-1β,6α-diol which were not eluted on GC.

2.2. Root oil

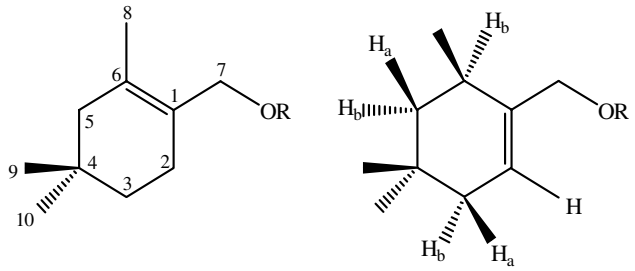
Analysis of the root oil by GC, GC–MS and ^{13}C NMR allowed the identification of nine components which represented nearly 45% of the composition, most of these compounds being already present in the leaf oil (Table 1). Once again, several compounds, including major components, remained unidentified. The oil was fractionated over silica gel into three fractions (olefins

Table 4
^{1}H and ^{13}C NMR spectral data, ^{1}H–^{1}H COSY and HMBC correlations for β-isocyclolavandulol **7**

C	δ (^{13}C)	DEPT	δ (^{1}H)[a]	Proton	Multiplicity (*J*, Hz)	^{1}H–^{1}H COSY	HMBC[b]
1	139.95	C	–	–	–	–	–
2	122.68	CH	5.63	–	*m*	3a, 3b, 7a, 7b	3, 4, 6, 7
3	39.28	CH_2	1.89	a	br *d* (17.5)	2, 3b, 6b, 7a, 7b, 9	1, 2, 4, 9
			1.77[c]	b	br *d* (17.5)	2, 3a, 5b, 7b	1, 2, 4, 5, 9
4	29.43	C	–	–	–	–	–
5	45.85	CH_2	1.11	a	*dd* (12.8;11.2)	5b, 6b, 9	1, 3, 4, 6, 8, 9, 10
			1.49	b	*ddd* (12.8; 5.6; 2.2)	3b, 5a, 6b	1, 3, 4, 6, 7, 8, 9, 10
6	28.56	CH	2.34	b	*m*	2, 3a, 5a, 5b, 8	1, 2, 5, 8
7	65.49	CH_2	4.05	a	*d* (12.7)	2, 3a, 7b	1, 2, 6
			4.19	b	br *d* (12.7)	2, 3a, 3b, 7a	1, 2, 6
8	19.13	CH_3	1.06	–	*d* (7.2)	6b	1, 5, 6
9	25.26	CH_3	0.89	–	*s*	3a, 5a, 10	3, 4, 5, 10
10	31.70	CH_3	0.96	–	*s*	9	3, 4, 5, 8, 9

[a] ^{1}H directly attached to ^{13}C determined from HSQC experiment.
[b] ^{1}H–^{13}C long-range correlation (HMBC) corresponding to two- or three-bond connectivities.
[c] Attribution carried out according to the correlation long-range with 5b.

R1, slightly polar oxygenated compounds R2 and alcohols R3). The slightly polar fraction R2 contained lavandulyl and β-cyclolavandulyl esters as well as an other series of monoterpene esters. Isolation of these esters by chromatography failed. Consequently, the fraction R2 was treated with $LiAlH_4$ and three alcohols were obtained. They were lavandulol (17%), β-cyclolavandulol **2** (27%) and an unidentified alcohol (42%), which was separated by combination of chromatography over silica gel and sephadex gel. This alcohol was submitted to structural analysis (MS, ^1H and ^{13}C NMR, 2D NMR: DEPT, COSY, HSQC and HMBC) (Table 4). The formula $C_{10}H_{18}O$ was deduced from the MS, ^1H, ^{13}C and DEPT spectra. The chemical shifts of protons and carbons indicated the presence of a trisubstituted double bond, a primary alcohol and three methyl groups (two singlets and a doublet). Obviously, the molecule bore a cyclic skeleton and the full set of carbon chemical shifts indicated that the alcohol could be an isomer of β-cyclolavandulol **2**. Examination of the two- and three-bond connectivities in the HMBC spectrum (Table 4) confirmed this point and compound **7** was identified as β-isocyclolavandulol (Fig. 1). Particularly, the connectivity between the protons H-7 and carbons C-1, C-2 and C-6 allowed the location of the oxygenated function with respect to the double bond. The corresponding esters, acetate **8**, propionate **9**, isobutyrate **10** and isovalerate **11** were prepared, their spectral data recorded (Tables 5 and 6) and subsequently identified in the fraction R2, in the root oil, and in the leaf oil (Table 1).



2-6 **7-11**

	R	
2	H	**7**
3	^{11}CO-$^{12}CH_3$	**8**
4	^{11}CO-$^{12}CH_2$-$^{13}CH_3$	**9**
5	^{11}CO-^{12}CH-$^{13,14}(CH_3)_2$	**10**
6	^{11}CO-$^{12}CH_2$-^{13}CH-$^{14,15}(CH_3)_2$	**11**

Fig. 1. Structure of *β*-cyclolavandulol, *β*-isocyclolavandulol and esters.

2.3. Antimicrobial activity

Results from antibacterial assays are summarised in Table 7. They show that root oil, and leaf oil in less degree, possessed an antibacterial effect on *Staphylococcus aureus*, *Serratia marcescens*, *Micrococcus luteus*, *Bacillus*

Table 6
^{13}C NMR spectral data of β-isocyclolavandulyl esters **8**–**11**

C	**8**	**9**	**10**	**11**
1	135.14	135.24	135.35	135.32
2	126.11	126.03	126.02	126.16
3	39.33	39.31	39.32	39.34
4	29.43	29.44	29.45	29.44
5	45.75	45.73	45.75	45.74
6	28.76	28.76	28.76	28.74
7	66.91	66.77	66.72	66.61
8	19.09	19.11	19.11	19.09
9	25.29	25.26	25.22	25.25
10	31.61	31.63	31.63	31.63
11	170.95	174.36	176.94	173.00
12	21.04	27.73	34.16	43.60
13		9.23	19.05*	25.74
14			19.00*	22.71*
15				22.42*

* Values may be reversed.

Table 5
^1H NMR spectral data of β-isocyclolavandulyl esters **8**–**11**

H	**8**	**9**	**10**	**11**
2	5.68; *m*	5.68; *m*	5.68; *m*	5.68; *m*
3a	1.90; br *d*	1.90; br *d*	1.89; br *d*	1.90; br *d*
3b	1.75; br *d*	1.78; br *d*	1.75; br *d*	1.74; br *d*
5a	1.10; *dd* (12.8; 11.4)	1.12; *dd* (12.7; 11.1)	1.10; *dd* (12.7; 11.0)	1.10; *dd* (12.8; 10.3)
5b	1.48; *ddd* (12.8; 5.7; 2.1)	1.49; *ddd* (12.7; 5.6; 2.0)	1.48; *ddd* (12.7; 5.4; 1.8)	1.48; *ddd* (12.8; 5.7; 2.1)
6b	2.28; *m*	2.18; *m*	2.28; *m*	2.28; *m*
7a	4.45; *d* (12.5)	4.49; *d* (12.5)	4.45; *d* (12.5)	4.45; *d* (12.5)
7b	4.65; *d* (12.5)	4.70; *d* (12.5)	4.65; *d* (12.5)	4.65; *d* (12.5)
8	1.02; *d* (7.5)	1.02; *d* (7.5)	1.02; *d* (7.2)	1.02; *d* (7.1)
9	0.90; *s*	0.88; *s*	0.88; *s*	0.87; *s*
10	0.95; *s*	0.95; *s*	0.95; *s*	0.93; *s*
12	2.07; *s*	2.35; *q* (7.5)	2.58; *h* (7.4)	2.20; *d* (7.2)
13		1.18; *t* (7.5)		2.08; *m*
13/14			1.18; *d* (7.4)	
14/15				0.97; *d* (7.2)

Table 7
Results of the disc diffusion assay (mm of inhibition growth)

Bacteria strains CIP No.	Ciprofloxacin	Penicillin G	DMSO	Root oil	Leaf oil
Bacillus maltophyla 60.77T	30	12	–	–	–
Bacillus subtilis 52.65T	44	31	–	18	20
Enterobacter cloacae 60.85T	41	–	–	–	–
Enterococcus faecalis 76117	28	29	–	14	20
Escherichia coli 58.4T	43	25	–	18	15
Micrococcus luteus 53.45	35	48	–	18	18
Proteus mirabilis A235	49	25	–	15	14
Pseudomonas aeruginosa A22	36	–	–	–	10
Serratia marcescens 58.14	45	–	–	20	16
Staphylococcus aureus 53.156	37	48	–	24	20
Staphylococcus epidermidis 53.124	28	–	–	16	–

subtilis, *Enterobacter cloacae* and *Escherichia coli*. The minimum inhibitory concentration (MIC) of root and leaf oils against *S. aureus* was 0.3% (3 mg/ml).

3. Conclusion

Combined analysis of *P. paniculatum* leaf and root oils, by CC, GC, GC–MS and ^{13}C NMR, led to the identification of 45 components which represented 87.4% and 81.3% of the whole composition, respectively. Both oils were dominated by irregular monoterpene esters bearing the lavandulyl or cyclolavandulyl skeletons. The main components of the leaf oil were β-cyclolavandulyl acetate and isobutyrate (16.1% and 17.8%, respectively) while the major components of the root oil were β-isocyclolavandulyl and β-cyclolavandulyl acetates (15.8% and 13.9%, respectively). Concerning the oxygenated cyclolavandulanes identified in both oils, only β-cyclolavandulal **1** has been previously isolated from a natural source (Logani et al., 1967). The two alcohols β-cyclolavandulol **2** and β-isocyclolavandulol **7** have been previously obtained by synthesis (Brenner and Schinz, 1952; Kuhn and Schinz, 1952; Oda et al., 1996). To the best of our knowledge, the β-cyclolavandulyl esters **3**–**6** and β-isocyclolavandulyl esters **8**–**11** are reported for the first time. Moreover, *P. paniculatum* leaf and root oils exhibited an antibacterial effect.

4. Experimental

4.1. Plant material

Leaves and roots of *P. paniculatum* L. were collected before flowering in April 2003 in a restricted area near Novella, Corsica, France. A voucher specimen has been deposited in the Herbarium of the Muséum d'Histoire Naturelle de la Ville de Nice, Nice, France, No. B-4617.

4.2. Hydrodistillation

Dried leaves (2621 g) and dried roots (1147 g) of *P. paniculatum* were individually hydrodistilled in a Clevenger type apparatus for 6 h. Leaf oil (4.4 g) and root oil (5.1 g) were obtained with 0.17% and 0.44% yield, respectively.

4.3. Chromatographic fractionation

Leaf oil (2.3 g) was fractioned by column chromatography on silica gel (ICN 63–200 μm, 80 g) and eluted with pentane and then Et_2O. A non-polar fraction L1 (pentane, 0.52 g) and an oxygenated fraction L2 (Et_2O, 1.77 g) were obtained. The fraction L2 was again fractioned by column chromatography on silica gel (SDS 35–70 μm, 50 g) eluted with a gradient of solvents (pentane:Et_2O = 97:3; 95:5; 75:25; 50:50; 0:100). Twenty-five fractions were obtained and gathered, according to the chromatographic profile, into 13 fractions L2.1–L2.13.

Root oil (3 g) was partitioned by column chromatography on silica gel (ICN 200–500 μm, 100 g). Three fractions were eluted: R1 (olefins, pentane, 0.121 g), R2 (slightly polar oxygenated compounds, pentane–Et_2O 90:10, 2.120 g) and R3 (alcohols, Et_2O, 0.539 g).

4.4. GC and GC–MS analysis

GC analysis was carried out using a Perkin–Elmer Autosystem GC apparatus equipped with two flame ionisation detectors and fused-silica columns, BP-1 (dimethylsiloxane) and BP-20 (polyethylene glycol) (50 m × 0.22 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 60 to 230 °C at 2 °C/min and then held isothermal for 35 min. The carrier gas was helium (0.8 ml/min). Injector and detector temperatures were 280 °C (injection mode:split 1:60). The relative percentage of the oil constituents was calculated from the GC peak areas without using correction factors. Retention indices (RI) of compounds were determined relative to the retention times of a series of

n-alkanes with linear interpolation (Target Compound Software from Perkin–Elmer).

GC–MS analysis was performed with a Hewlett-Packard 5890 chromatograph coupled with a 5970A mass spectrometer of the same company, using the following conditions: fused-silica capillary column HP-1 (dimethylsiloxane) 50 m × 0.2 mm i.d., film thickness 0.5 μm. Injection mode:split 1:30. The oven temperature was programmed from 60 to 250 °C at 2 °C/min and then held isothermal for 30 min. The carrier gas was helium (0.8 ml/min). Injector and detector temperatures were 250 and 230 °C, respectively. The mass spectra were performed at 70 eV, mass range 35–400. Components were identified by comparison of their mass spectra with those compiled in commercial database and laboratory-made spectral libraries, as well as by comparison of their retention indices with those of authentic samples or literature data.

4.5. NMR

The NMR spectra (^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC) of pure compounds were recorded on a Bruker AMX-400 or Bruker AC-200 instruments, in $CDCl_3$, using the Bruker micro programs. The chemical shift values are reported with reference to TMS and the coupling constants are given in Hertz.

The ^{13}C NMR spectra of mixtures (essential oil or fraction of chromatography) were recorded on a Bruker AC-200 instrument, equipped with a 5 mm probe, with the following parameters: pulse width (PW), 3.2 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. The number of accumulated scans was 5000–10,000 for each sample (approximately 70 mg of mixture in 0.5 ml $CDCl_3$).

4.6. β-Isocyclolavandulol (7)

The fraction F2 (2 g) dissolved in 20 ml dry Et_2O was carefully added to a suspension of $LiAlH_4$ (533 mg) in 30 ml dry Et_2O at 0 °C. The mixture was stirred at room temperature and then refluxed for 3 h. The reaction mixture was hydrolysed by addition of a solution of 15% NaOH (2 ml) and cold water. The organic layer was separated, washed with water to neutrality, dried over Na_2SO_4 and conc. in vacuum. The mixture (1.36 g) contained three alcohols as major components: lavandulol (17%), β-cyclolavandulol (27%) and β-isocyclolavandulol (42%, characterised after isolation). Then, β-cyclolavandulol was removed from the mixture by chromatography on silica gel (SDS 60 ACC chromagel 35–70 μm; 100 g, elution with pentane:Et_2O = 30:70). Final purification was achieved by chromatography on sephadex LH-20 (15 g, elution with CH_2Cl_2) to give β-isocyclolavandulol **7** (135 mg, 98% purity on GC) as a colourless oil. ^{1}H NMR and ^{13}C NMR data (400 MHz, $CDCl_3$), see Table 4. EIMS 70 eV, *m*/*z* (rel. int.): 154 [M^+] (33), 136 (29), 123 (100), 121 (55), 95 (43), 93 (63), 83 (45), 81 (53), 80 (81), 79 (60), 69 (44), 55 (33), 41 (43).

4.7. β-Isocyclolavandulyl acetate (8)

A solution of β-isocyclolavandulol (**7**) (30 mg; 0.19 mmol), pyridine (5 ml) and Ac_2O (5 ml) was stirred overnight at room temperature. Then, water (20 ml) was added and the mixture was extracted with Et_2O (3 × 20 ml). The organic layer was successively washed with a saturated solution of $CuSO_4$ (3 × 20 ml), a saturated solution of Na_2CO_3 (3 × 20 ml), with water until neutral pH, then dried on Na_2SO_4 and conc. in vacuum to yield β-isocyclolavandulyl acetate **8** as a colourless oil (36.5 mg, 0.186 mmol, 95% yield, 97% purity on GC). ^{1}H NMR and ^{13}C NMR data (400 MHz, $CDCl_3$), see Tables 5 and 6. EIMS 70 eV, *m*/*z* (rel. int.): 136 (36), 121 (100), 107 (39), 94 (21), 93 (79), 91 (16), 81 (12), 80 (25), 79 (35), 67 (8), 55 (9), 43 (35), 41 (18).

4.8. General procedure for esterification of 2 and 7

To a solution of alcohol (1 equiv.) in 30 ml CH_2Cl_2 was added Et_3N (1.5 equiv.), and the mixture was cooled to 0 °C. A solution of acid chloride (2.5 equiv., in 30 ml CH_2Cl_2) was added drop by drop and the mixture was refluxed during 3 h. The mixture was poured into 200 ml of cold water. After decantation, the organic phase was washed twice with water, dried on Na_2SO_4 and conc. in vacuum. The crude ester was purified by CC on silica gel. Yields were not optimised.

4.8.1. β-Cyclolavandulyl acetate (3)

2: 150 mg (0.97 mmol); Et_3N: 130 mg (1.29 mmol); acetyl chloride: 122 mg (1.78 mmol). Compound **3**: 145 mg, 0.74 mmol, 78% yield, colourless oil, ^{1}H NMR and ^{13}C NMR data (200 MHz, $CDCl_3$), see Tables 2 and 3. EIMS 70 eV, *m*/*z* (rel. int.): 136 (36), 121 (100), 107 (38), 95 (11), 94 (20), 93 (90), 91 (17), 79 (25), 77 (12), 55 (8), 43 (32), 41 (18).

4.8.2. β-Cyclolavandulyl propionate (4)

2: 150 mg (0.97 mmol); Et_3N: 131 mg (1.30 mmol); propionyl chloride: 166 mg (1.79 mmol). Compound **4**: 121 mg, 0.60 mmol, 62% yield, colourless oil, ^{1}H NMR and ^{13}C NMR data (200 MHz, $CDCl_3$), see Tables 2 and 3. EIMS 70 eV, *m*/*z* (rel. int.): 137 (12), 136 (38), 121 (100), 107 (41), 94 (21), 93 (88), 91 (13), 79 (19), 67 (7), 57 (22), 41 (13).

4.8.3. β-Cyclolavandulyl isobutyrate (5)

2: 153 mg (0.99 mmol); Et_3N: 162 mg (1.61 mmol); isobutyryl chloride: 165 mg (1.55 mmol). Compound **5**:

43 mg, 0.20 mmol, 20% yield, colourless oil, ^{1}H NMR and ^{13}C NMR data (200 MHz, $CDCl_3$), see Tables 2 and 3. EIMS 70 eV, *m/z* (rel. int.): 137 (11), 136 (41), 122 (10), 121 (100), 108 (10), 107 (41), 95 (12), 94 (21), 93 (71), 91 (11), 81 (14), 80 (17), 79 (23), 77 (9), 71 (14), 43 (26), 41 (20).

4.8.4. β-Cyclolavandulyl isovalerate (**6**)

2: 151 mg (0.98 mmol); Et_3N: 146 mg (1.45 mmol); isovaleryl chloride: 244 mg (2.02 mmol). Compound **6**: 63 mg, 0.28 mmol, 29% yield, colourless oil, ^{1}H NMR and ^{13}C NMR data (200 MHz, $CDCl_3$), see Tables 2 and 3. EIMS 70 eV, *m/z* (rel. int.): 136 (48), 121 (100), 107 (43), 94 (22), 93 (74), 85 (17), 80 (18), 79 (19), 57 (20), 41 (15).

4.8.5. β-Isocyclolavandulyl propionate (**9**)

7: 25 mg (0.16 mmol); Et_3N: 27 mg (0.27 mmol); propionyl chloride: 25 mg (0.27 mmol). Compound **9**: 23 mg, 0.11 mmol, 68% yield, colourless oil, ^{1}H NMR and ^{13}C NMR data (400 MHz, $CDCl_3$), see Tables 5 and 6. EIMS 70 eV, *m/z* (rel. int.): 137 (9), 136 (39), 122 (10), 121 (100), 108 (10), 107 (40), 95 (9), 94 (21), 93 (76), 91 (12), 81 (11), 80 (21), 79 (26), 77 (9), 57 (26), 41 (12).

4.8.6. β-Isocyclolavandulyl isobutyrate (**10**)

7: 30 mg (0.19 mmol); Et_3N: 30 mg (0.30 mmol); isobutyryl chloride: 31 mg (0.29 mmol). Compound **10**: 21 mg, 0.094 mmol, 48% yield, colourless oil, ^{1}H NMR and ^{13}C NMR data (400 MHz, $CDCl_3$), see Tables 5 and 6. EIMS 70 eV, *m/z* (rel. int.): 136 (17), 121 (40), 107 (13), 94 (13), 93 (100), 92 (16), 80 (23), 71 (38), 69 (76), 68 (36), 67 (14), 53 (7), 43 (31), 41 (29).

4.8.7. β-Isocyclolavandulyl isovalerate (**11**)

7: 30 mg (0.19 mmol); Et_3N: 33 mg (0.33 mmol); isovaleryl chloride: 59 mg (0.490 mmol). Compound **11**: 32 mg, 0.135 mmol, 70% yield, colourless oil, ^{1}H NMR and ^{13}C NMR data (400 MHz, $CDCl_3$), see Tables 5 and 6. EIMS 70 eV, *m/z* (rel. int.): 136 (47), 121 (100), 107 (42), 94 (22), 93 (71), 81 (13), 80 (16), 79 (19), 57 (29), 41 (17).

4.9. Antimicrobial screening

Essential oils were individually tested against eleven bacterial strains: *Bacillus maltophyla* 60.77T; *B. subtilis* 5265T; *E. cloacae* 60.85T; *Enterococcus faecalis* 76117; *E. coli* 54.8T; *M. luteus* 5345; *Proteus mirabilis* A235; *Pseudomonas aeruginosa* A22; *S. marcescens* 5814; *S. aureus* 53156; *Staphylococcus epidermidis* 53124; (Pasteur Institute). The agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oils. A suspension of the tested microorganism (0.1 ml of 10^8 cells/ml) was spread onto the surface of Mueller Hinton Agar plates. Filter paper discs (6 mm in diameter) were soaked with 15 μl of filtered-sterilised fractions using a 0.45 μm membrane filter and 5 μl of DMSO and placed on the surface of inoculated plates. Ciprofloxacin (5 μg) and penicillin (6 μg) were used as positive controls and DMSO as negative one. After incubation at 37 °C for 24 h, the diameters of the inhibition zones were measured (mm). Each test was carried out in triplicate.

4.10. Determination of minimum inhibitory concentrations

A concentration range of tested oils in Mueller Hinton medium was done (0.1–0.6%). 10 μl of a standardised suspension (5×10^4 UFC) was added. Inoculated plates were incubated at 37 °C for 24 h. The MIC was defined as lowest concentration of tested samples that resulted in a complete inhibition of visible growth.

References

Ahmad, V.U., Faroqui, T.A., Fizza, K., Sultana, A., Khatoon, R., 1992. Tree new eudesmane sesquiterpenes from *Pluchea arguta*. J. Nat. Prod. 55, 730–735.

Aubert, J., Descimon, H., Michel, F., 1996. Population biology and conservation of the Corsican swallowtail butterfly *Papilio hospiton* Géné. Biol. Conserv. 78, 247–255.

Brenner, A., Schinz, H., 1952. Über einige β-Cyclolavandulyl-Verbindungen. Helv. Chim. Acta 35, 1615–1626.

Formácek, V., Kubeczka, K.H., 1982. Essential Oil Analysis by Capillary Gas Chromatography and Carbon-13 NMR Spectroscopy. Wiley, Chichester, UK.

Gamisans, J., Jeanmonod, D., 1993. In: Jeanmonod, D., Burdet, H.M. (Eds.), Catalogue des plantes vasculaires de la Corse, Geneva, Switzerland.

Gamisans, J., Marzocchi, J.F., 1996. La flore endémique de la Corse. Edisud, Aix-en-Provence, France.

Kesselmans, R.P.W., Wijnberg, J.B.P.A., 1992. Chromatographic and spectroscopic data of all stereoisomers of eudesm-11-en-4-ol. J. Essent. Oil Res. 4, 201–217.

Kuhn, W., Schinz, H., 1952. Über die Lavandulylsäure und ihre Umwandlungsprodukte. Helv. Chim. Acta 35, 2008–2015.

Logani, M.K., Varshney, I.P., Pandey, R.C., Dev, S., 1967. β-Cyclolavandulal, a new naturally occurring monoterpene type. Tetrahedron Lett., 2645–2648.

Oda, M., Isobe, A., Hayashi, M., Miyatake, R., Shimao, I., Kuroda, S., 1996. An efficient synthetic method for β-cyclolavandulal and its corresponding alcohol. Rec. Trav. Chim. Pays-Bas 115, 438–440.

Rezzi, S., Bighelli, A., Castola, V., Casanova, J., 2002. Direct identification and quantitative determination of acidic and neutral diterpenes using ^{13}C-NMR spectroscopy. Application to the analysis of oleoresin of *Pinus nigra*. Appl. Spectrosc. 56, 312–317.

Su, W.C., Fang, J.M., Cheng, Y.S., 1995. Sesquiterpenes from leaves of *Cryptomeria japonica*. Phytochemistry 39, 603–607.

Tomi, F., Bradesi, P., Bighelli, A., Casanova, J., 1995. Computer-aided identification of individual components of essential oils using carbon-13 NMR spectroscopy. J. Magn. Reson. Anal. 1, 25–34.