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Composition, irregular terpenoids, chemical variability and antibacterial activity of the essential oil from *Santolina corsica* Jordan et Fourr

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Abstract

A detailed analysis of *Santolina corsica* essential oil was carried out by combination of GC (RI), GC–MS and ^{13}C NMR spectroscopy. After fractionation by column chromatography, 50 components were identified, accounting for 88.2% of the total amount of the oil. The chemical composition was dominated by monoterpene hydrocarbons, myrcene (34.6%), santolina triene (13.5%) and β-phellandrene (11.7%). Beside the main compounds, we noted the occurrence of irregular mono and sesquiterpenes belonging to five families: santolinane, artemisane, chrysanthemane, lavandulane and sesquilavandulane. Three compounds, lyratyl butyrate, isolyratone and *epi*-isolyratol were isolated and their structure elucidated by 2D NMR. Antibacterial activity was tested against six bacteria strains. The essential oil was effective against *Staphylococcus aureus* and *C. jejuni*. Lyratol was identified as main responsive of the antibacterial activity. The content of lyratol was measured in 33 oil samples isolated from individual plants.

Keywords: *Santolina corsica*; Essential oil composition; Irregular monoterpenes; Santolina triene; Lyratol; Lyratyl butyrate; *epi*-Isolyratol; Isolyratone; Antibacterial activity

1. Introduction

The genus *Santolina* is represented by more than 10 species widely distributed in Mediterranean area (Derbesy et al., 1989). Among *Santolina* species, *S. viridis* W. (south of France and north of Spain), *S. pectinata* Lag. (Iberian peninsula) and *S. chamaecyparissus* L. (which grows wild all around the Mediterranean basin) are the most widespread. *Santolina corsica* Jordan et Fourr. is an endemic species to Corsica and Sardinia (Arrigoni, 1979). It is an under-shrub, 30–50 cm high, with persistent leaves and yellow flowers. It grows preferably on the rocky and sunny places.

The composition of essential oils from various species of *Santolina* has been investigated: *S. chamaecyparissus* (Lawrence, 1997; Garg et al., 2001), *S. oblongifolia* (De Pascual et al., 1983), *S. ligustica* (Flamini et al., 1999), *S. rosmarinifolia* (Palà-Paùl et al., 1999, 2001) and *S. canescens* (Casado et al., 2001). All the species produced monoterpene-rich oils and exhibited quite diverse compositions. Conversely, only three studies reported on the phytochemistry of *S. corsica*. A solvent extract from roots contained sesquiterpene hydrocarbons, triterpenes, furylthienylbutenynes and a spiroketalenol (Ferrari et al., 2005). The composition of the essential oil of *S. corsica* was briefly reported. The Sardinian oil sample was dominated by camphor (18.5%), artemisia ketone (12.9%) and borneol (7.4%)

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(Poli et al., 1997) while the oil of Corsican origin contained artemisia ketone (20.0%), β-phellandrene (14.4%), myrcene (11.7%) and santolina triene (8.2%) as main components. The Corsican oil exhibited an appreciable antibacterial activity against *Staphylococcus aureus* (Rossi et al., 2007).

The aim of the present study was to get a better insight on the composition and the antibacterial activity of the essential oil from *S. corsica*. Consequently, an oil sample, particularly active against *S. aureus* and *Campylobacter jejuni* was analysed by combination of complementary analytical techniques after fractionation on column chromatography, allowing the isolation of the active compound and of three new irregular monoterpenoids whose structure was elucidated. Finally a study on the chemical variability of the essential oil from *S. corsica* was carried out on individual plants, in order to check the content of the active compound and the usefulness of the plant.

2. Results and discussion

2.1. Chemical analysis and structure elucidation of new compounds

The essential oil was isolated from the aerial parts of *S. corsica* by hydrodistillation (yield = 0.34%, w/w *vs.* fresh material). It resulted from the direct analysis of the oil by GC(RI), GC–MS and ^{13}C NMR (computer matching against commercial and laboratory-built libraries), that several compounds remained unidentified. Consequently, the bulk sample was submitted to repeated chromatography on silica gel and all the fractions were analysed by GC(RI), GC–MS and ^{13}C NMR. In total, 50 compounds were identified, accounting for 88.2% of the oil (Table 1). Most compounds were identified by comparison of their spectral data (MS and/or NMR) with those of authentic compounds compiled in our laboratory-made mass and ^{13}C NMR spectra libraries. A few compounds were identified using the literature data (see Section 3). Otherwise, lyratal **29**, lyratyl butyrate **40** and lyratyl isovalerate **44** were identified by comparison of their MS and ^{13}C NMR data with those of authentic samples prepared by oxidation or esterification of lyratol **30**, isolated from essential oil (see Fig. 1). The occurrence of lyratal **29** and lyratyl isovalerate **44** was mentioned in the essential oil of *Chrysanthemum coronarium* (Bohlmann and Fritz, 1979) and *Artemisia vulgaris* (Näf-Muller et al., 1981), respectively, while lyratyl butyrate **40** is reported for the first time.

Two compounds (accounting for 1.3% and 1.0% of the oil, respectively) remained unidentified. The first one, compound **23** (RI = 1042 and 1518) exhibited the same mass spectrum as isolyratol **25**, (RI = 1052/1557). Although the oxygenated fraction was submitted to repeated chromatography on silica gel and Sephadex, compound **23** could not be obtained in a pure form. However, it was possible to carry out the structural analysis from the 1D and 2D NMR spectra (^{1}H NMR, ^{13}C NMR, DEPT, COSY, HSQC and HMBC) of a fraction of chromatography which contained isolyratol **25** and the unidentified compound **23** (**25/23** = 5/2). We first identified all the signals belonging to isolyratol (Epstein and Gaudioso, 1984; Ferreira et al., 1998) as well as the correlation plots in the homonuclear and heteronuclear 2D spectra (Table 2). The structure of compound **23** was deduced from the remaining signals and correlation plots. Indeed, the chemical shifts of protons and carbons indicated the presence of three double bonds, a hydroxyl group, two methyl groups and one methine (DEPT). Then, the structure of **23**, which possess the santolina triene skeleton, was deduced from the HMBC spectrum (Table 2) and we suggest the name of *epi*-isolyratol for that compound. Although this epimer of lyratol is reported for the first time, it should be mentioned that the corresponding hydroperoxide was isolated from *Artemisia lamcea* (Luo et al., 1991).

The diastereoisomeric relation between *epi*-isolyratol **23** and isolyratol **25** was unambiguously established by oxidation of a mixture of **23** and **25** leading to an unique ketone, 2,5-dimethyl-4-vinyl-2,5-hexadien-3-one. The structure of that ketone, reported for the first time and named isolyratone, was confirmed by structural analysis (MS, ^{1}H and ^{13}C NMR, DEPT, COSY, HSQC and HMBC spectra) (Table 3). Then, it was easy to identify isolyratone **16**, in the fraction of chromatography and in the essential oil (1.0%).

The composition of the investigated sample of *S. corsica* essential oil is dominated by monoterpene hydrocarbons, myrcene (34.6%), santolina triene (13.5%) and β-phellandrene (11.7%). Beside the main compounds, we note the occurrence of regular monoterpenes, a few sesquiterpenes and overall, several irregular oxygenated mono and sesquiterpenes (about 11% of the oil). It should be pointed out that these irregular compounds belong to five families: santolinane (santolina triene **4**, isolyratone **16**, *epi*-isolyratol **23**, isolyratol **25**, lyratal **29**, lyratol **30** and its esters **38**, **40** and **44**), artemisane (yomogi alcohol **13**, artemisia ketone **22**, artemisia alcohol **26**), chrysanthemane (*trans* and *cis* chrysanthemyl alcohols **31** and **32**), lavandulane (lavandulol **33**) and sesquilavandulane (3,9-dimethyl-6-isopropyl-2*Z* and 2*E*,7*E*,9-decatrienal **49** and **50**).

2.2. Antimicrobial activity, active compound

The antimicrobial activity of the same oil sample of *S. corsica* was tested, *in vitro*, by using the agar diffusion method with six microorganisms, as seen in Table 4. The DMSO (10%) did not show antibacterial activity against the tested bacteria (negative control). The growth of *P. aeruginosa*,*E. aerogenes*, and *E. coli* was not inhibited by the essential oil. The activity against *L. innocua* was moderate (9.5 mm), while the growth of *S. aureus* (14.7 mm) and especially *C. jejuni* (39 mm) was well inhibited.

In order to determine the antibacterial compounds, we tested the hydrocarbonated and the oxygenated fractions, separated by column chromatography on silica gel, against

Table 1
Composition of *Santolina corsica* essential oil

	Constituent[a]	RI A	RI P	%	Identification
1	(*Z*)-Hex-3-en-1-ol	832	1384	0.1	RI, MS, ^{13}C NMR
2	(*E*)-Hex-2-en-1-ol	842	1406	0.1	RI, MS, ^{13}C NMR
3	Hexanol-1	845	1349	0.1	RI, MS, ^{13}C NMR
4	Santolina triene	901	1030	13.5	RI, MS, ^{13}C NMR
5	α-Thujene	923	1027	tr	RI, MS
6	Benzaldelyde	929	1529	tr	RI, MS
7	α-Pinene	931	1025	0.3	RI, MS, ^{13}C NMR
8	Camphene	943	1070	tr	RI, MS
9	Sabinene	965	1124	2.6	RI, MS, ^{13}C NMR
10	β-Pinene	970	1113	3.5	RI, MS, ^{13}C NMR
11	1,8-Dehydro-cineol	979	1191	tr	RI, MS, ^{13}C NMR
12	Myrcene	982	1166	34.6	RI, MS, ^{13}C NMR
13	Yomogi alcohol	984	1400	0.2	RI, MS, ^{13}C NMR
14	3-Carene	1005	1152	tr	RI, MS
15	*para*-Cymene	1011	1265	0.1	RI, MS, ^{13}C NMR
16	Isolyratone	1014	1368	1.0	RI, MS, ^{1}H, ^{13}C, 2D NMR
17	Limonene*	1021	1203	1.5	RI, MS, ^{13}C NMR
18	β-Phellandrene*	1021	1214	11.7^{+}	RI, MS, ^{13}C NMR
19	1,8-Cineole*	1021	1214	2.0^{+}	RI, MS, ^{13}C NMR
20	Santolina alcohol	1026	1402	tr	RI, MS, ^{13}C NMR
21	(*Z*)-β-Ocimene	1029	1251	tr	RI, MS
22	Artemisia ketone*	1042	1349	0.1	RI, MS, ^{13}C NMR
23	*epi*-Isolyratol*[b]	1042	1518	1.3	RI, MS, ^{1}H, ^{13}C, 2D NMR
24	γ-Terpinene	1048	1247	0.5	RI, MS, ^{13}C NMR
25	Isolyratol	1052	1557	2.8	RI, MS, ^{13}C NMR
26	Artemisia alcohol	1067	1510	0.1	RI, MS, ^{13}C NMR
27	Terpinolene	1078	1285	1.0	RI, MS, ^{13}C NMR
28	Linalool	1084	1545	0.1	RI, MS, ^{13}C NMR
29	Lyratal	1097	1505	0.5	RI, MS, ^{13}C NMR
30	Lyratol	1138	1784	6.1	RI, MS, ^{13}C NMR
31	*trans*-Chrysanthemyl alcohol	1141	1677	tr	RI, MS
32	*cis*-Chrysanthemyl alcohol	1146	1660	tr	RI, MS
33	Lavandulol	1149	1674	0.1	RI, MS, ^{13}C NMR
34	Cryptone	1155	1673	0.1	RI, MS, ^{13}C NMR
35	Terpinen-4-ol	1162	1600	1.4	RI, MS, ^{13}C NMR
36	α-Terpineol	1172	1698	0.2	RI, MS, ^{13}C NMR
37	Estragole	1174	1670	tr	RI, MS
38	Lyratyl acetate	1250	1647	0.4	RI, MS, ^{13}C NMR
39	7-α-Silpherfol-5-ene	1327	1426	tr	RI, MS
40	Lyratyl butyrate	1425	1785	tr	RI, MS, ^{13}C NMR
41	*allo*-Aromadendrene	1460	1645	0.2	RI, MS, ^{13}C NMR
42	α-Curcumene	1470	1769	0.2	RI, MS, ^{13}C NMR
43	γ-Curcumene	1472	1682	1.0	RI, MS, ^{13}C NMR
44	Lyratyl isovalerate	1478	1806	tr	RI, MS, ^{13}C NMR
45	Bicyclogermacrene	1492	1734	0.2	RI, MS, ^{13}C NMR
46	(*E*)-Nerolidol	1545	2038	tr	RI, MS, ^{13}C NMR
47	Spathulenol	1572	2115	tr	RI, MS, ^{13}C NMR
48	Caryophyllene oxide	1580	1984	tr	RI, MS, ^{13}C NMR
49	3,9-Dimethyl-6-isopropyl-2*Z*,7*E*,9-decatrienal*	1639	215	0.2	RI, MS, ^{13}C NMR
50	3,9-Dimethyl-6-isopropyl-2*E*,7*E*,9-decatrienal*	1639	2155	0.4	RI, MS, ^{13}C NMR
	Total (%)			88.2	

RI A, RI P: retention indices on apolar and polar column, respectively; tr. ⩽ 0.05%.
[a] Order of elution and percentages (%) of individual components are given on apolar column except for compounds marked* (percentages given on polar column).
[b] Identified by 1D and 2D NMR. + Percentages (%) of β-phellandrene and 1,8-cineole were given by taking accounts of the intensity of NMR spectra.

S. aureus and *C. jejuni* (Table 5). As expected, the hydrocarbonated fraction exhibited a weak activity (inhibition zone diameter = 8.5 mm) against *S. aureus*. Indeed, myrcene which accounted for 57.2% in the fraction was already reported as ineffective against *S. aureus* (Wannissorn et al., 2005). The inhibition diameter was of 27.5 mm against *C. jejuni* which is known as very sensible to essential oil (Angioni et al., 2003). Conversely, a significant antibacterial

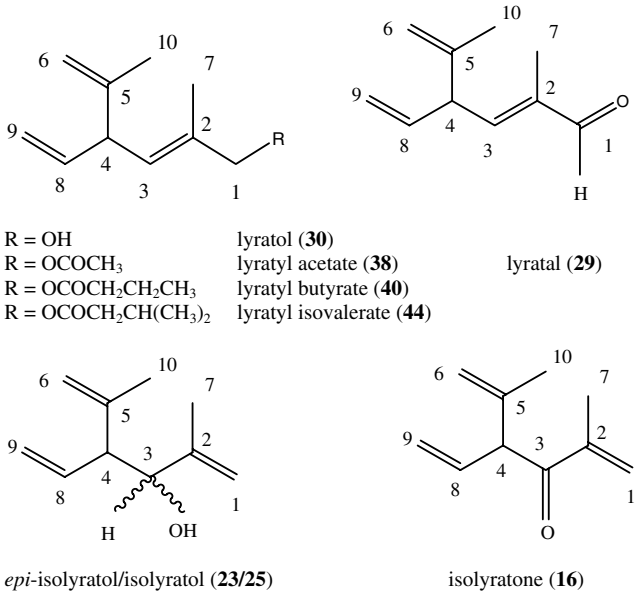

Fig. 1. Structures of compounds **16**, **23**, **25**, **29**, **30**, **38**, **40**, and **44**.

activity was observed with the polar fraction against *S. aureus* (inhibition diameter = 17.7 mm). The higher level of activity of the polar fraction was demonstrated for *C. jejuni* with an inhibition diameter of 62.6 mm. Thus, we focused our study on that oxygenated fraction, characterised by the pre-eminence of lyratol (27.9%), 1,8-cineole (10.8%) and isolyratol (9.2%). 1,8-Cineole was previously described as antibacterial against *S. aureus* (Santoyo et al., 2005) and moderately antibacterial against *C. jejuni* (Friedman et al., 2002) but its low amount (2%) observed in the essential oil could not explain such an activity and suggested that other antibacterial compounds were present. Indeed, the antibacterial activity of a lyratol-rich fraction (84%), obtained after repeated chromatography and totally devoid in 1,8-cineole, was tested. An antibacterial activity was observed, significant against *S. aureus* (inhibition diameter = 19 mm) and particularly high against *C. jejuni* (inhibition diameter = 90 mm), suggesting that lyratol was the main responsive of the *S. corsica* antimicrobial activity.

2.3. Chemical variability

As a general rule, irregular monoterpenes are frequently encountered in *Santolina* oils: artemisia ketone (sometimes up to 60% of the chemical composition of the oil), artemisia alcohol, artemisia triene, santolina triene, santolina alcohol, yomogi alcohol, chrysanthemyl alcohols and sometimes lavandulol (Lawrence, 1991, 1992, 1997; Palà-Paùl et al., 1999; Cherchi et al., 2001). To our knowledge the presence of lyratol and lyratyl derivatives as well as sesquilavandulane derivatives has been only reported by our group in *Santolina* oils. Otherwise, the chemical composition of the investigated oil sample, differed drastically from that of the sample from Sardinia (Poli et al., 1997). It also varied substantially from the composition of the previously investigated sample from Corsica which contained artemisia ketone as main component (Rossi et al., 2007).

Table 2
^{1}H and ^{13}C NMR spectral data and HMBC correlation for **23** and **25**

C	$\delta(^{13}C)$	DEPT	$\delta(^{1}H)$[a]	Mutiplicity (*J*, Hz)	HMBC[b]
Compound **25** isolyratol					
1	113.21	CH_2	4.96; 4.90	*m*	2,3,7
2	144.86	C	–	–	–
3	75.86	CH	4.15	*d* (8.4)	1,2,4,5,7,8
4	56.11	CH	2.90	*t* (8.4)	2,3,5,6,8,9,10
5	144.76	C	–	–	–
6	113.05	CH_2	4.81; 4.85	*m*	3,4,5,10
7	17.36	CH_3	1.74	*dd* (1.4; 0.8)	1,2,3,4
8	136.83	CH	5.92	*ddd* (17.0; 10.2; 8.4)	2,3,4,5
9	117.78	CH_2	5.18	*ddd* (17.0; 1.7; 0.7)	4,8
			5.21	*ddd* (10.2; 1.7; 0.7)	4,8
10	20.65	CH_3	1.75	*dd* (1.2; 1.0)	4,5,6
Compound **23** *epi*-isolyratol					
1	114.39	CH_2	4.95; 4.90	*m*	2,3,7
2	144.74	C	–	–	–
3	76.07	CH	4.09	*d* (8.4)	1,2,4,5,7,8
4	56.20	CH	2.90	*t* (8.4)	2,3,5,6,8,9,10
5	145.01	C	–	–	–
6	113.90	CH_2	4.93; 4.97	*m*	3,4,5,10
7	16.63	CH_3	1.72	*dd* (1.6; 0.8)	1,2,3,4
8	136.25	CH	5.68	*ddd* (17.2; 10.1; 8.4)	2,3,4,5
9	116.28	CH_2	5.07	*ddd* (17.2; 1.6; 1.3)	4,8
			5.04	*ddd* (10.1; 1.6; 0.7)	4,8
10	19.64	CH_3	1.78	*dd* (1.6; 0.8)	4,5,6

[a] ^{1}H directly attached to ^{13}C determined from HSQC experiment.
[b] ^{1}H–^{13}C long-rang correlation (HMBC) corresponding to two- or three-bond connectivities.

Table 3
^{1}H and ^{13}C NMR spectral data, ^{1}H–^{1}H COSY and HMBC correlation for isolyratone **16**

C	$\delta(^{13}C)$	DEPT	$\delta(^1H)$[a]	Proton	Multiplicity (*J*, Hz)	^{1}H–^{1}H COSY	HMBC[b]
1	124.86	CH_2	6.02	a	broad *s*	1b, 7	2, 3, 7
			5.77	b	*m*	1a, 7	2, 3, 7
2	143.86	C	–		–	–	–
3	200.39	C	–		–	–	–
4	58.44	CH	4.40		broad *d* (8.1)	8	3, 5, 6, 8, 9, 10
5	143.24	C	–		–	–	–
6	113.73	CH_2	4.93	a	*quint* (1.4)	6b, 10	4, 5, 10
			4.81	b	broad *s*	6a, 10	4, 5, 10
7	18.07	CH_3	1.89		*dd* (1.4, 1.1)	1a, 1b	1, 2, 3
8	135.68	CH	6.07		*ddd* (17.2, 10.2, 8.1)	4, 9	4
9	117.01	CH_2	5.17	a	*ddd* (10.2, 1.4, 0.9)	9b, 8	4, 8
			5.10	b	*dt* (17.2, 1.4)	9a, 8	4, 8
10	21.18	CH_3	1.74		*dd* (1.4, 1.0)	6a, 6b	4, 5, 6

[a] ^{1}H directly attached to ^{13}C determined from HSQC experiment.
[b] ^{1}H–^{13}C long-rang correlation (HMBC) corresponding to two- or three-bond connectivities.

Table 4
Antibacterial activity of *Santolina corsica* essential oil

Samples	*P. aeruginosa*	*E. aerogenes*	*E. coli*	*L. innocua*	*S. aureus*	*C. jejuni*
Essential oil	6	6.4	6.6	9.5	14.7	39.0
Antibiotic	34.2	29.7	34.6	34.3	41.8	35.0
Negative control	6	6	6	6	6	6

Inhibition diameters were given in mm. disc included, DMSO 10% in water was used as negative control. Antibiotics were, respectively penicillin G, rifampicine, and amikacine for *S. aureus*. *L. innocua* and *C. jejuni*, for *E. coli*, *P. aeruginosa*, and *E. aerogenes* ciprofloxacine was used.

Table 5
Antibacterial activity of *S. corsica* essential oil separated fractions

Samples	*S. aureus*	*C. jejuni*
Hydrocarbonated fraction	8.5	27.5
Oxygenated fraction	17.7	62.6
Lyratol-rich fraction (84%)	19	90
Negative control	6	6

Inhibition diameter are given in mm, disc included, DMSO 10% in water was used as negative control.

In order to have an overview on the composition of the essential oil from *S. corsica*, and more precisely on the content of lyratol and lyratyl derivatives, 33 individual plants were harvested in the principal locations around the city of Corte (centre of Corsica). The plants were separately hydrodistilled, the essential oils were analysed by GC (RI) and ^{13}C NMR and the results were submitted to K-means and PCA analysis. However, statistical analysis did not succeed to distribute the 33 compositions into clearly defined groups. All the samples, one excepted, were characterized by the association of santolina triene (9.3–42.9%), myrcene (6.1–34.6%) and β-phellandrene (3.3–26.9%) as main components. Sabinene and β-pinene accounted for 1.4–7.2% and 1.6–7.1%, respectively. Among irregular monoterpenes, the active compound lyratol **30** represented 5.3–10.3% of the whole composition for 19 samples out of 33. The percentage was lower for 10 oil samples (2.3–4.5%) and very low for two samples (0.3% and 1.5%). Conversely, it reached 11.8% and 14.4% for the two last samples. The content of the other irregular monoterpenes varied substantially: yomogi alcohol **13** (0–3.7%), isolyratone **16** (0.4–2.3%), *epi*-isolyratol **23** (0.7–3.0%), isolyratol **25** (1.6–6.4%), artemisia alcohol **26** (0–2.4%), lyratal **29** (0.1–1.7%), lavandulol **33** (0–0.5%), lyratyl acetate **38** (0.1–1.9%), lyratyl butyrate **40** (0–0.4%), lyratyl isovalerate **44** (0–0.2%), (*Z*) and (*E*)-sesquilavandulals **49** and **50** (0–0.9% and 0.1–0.8%).

One oil sample presented, beside myrcene (24.1%), β-phellandrene (11.9%) and santolina triene (9.6%), a noticeable amount of artemisia ketone (18.4%). This compound accounted for 1.2–4.5% in four samples out of 33, whilst its percentage did not exceed 0.5% in the other samples. It can be conclude that the artemisia ketone-rich composition is not representative of the *S. corsica* essential oil and should be considered as an atypical composition. No oil sample from Corsica exhibited a composition close to that reported for a Sardinian oil (Poli et al., 1997).

3. Experimental

3.1. Plant material and essential oil preparation

The aerial parts of 33 individual plants of *S. corsica* were collected at the flowering stage in June 2005 near Corte (Corsica, France). The essential oils were obtained by hydrodistillation with a Clevenger-type apparatus for 4 h. The

yields calculated from fresh-material ranged from 0.33% to 0.60%. A collective oil sample was obtained by hydrodistillation of several plants and used for chromatographic partitioning.

3.2. Oil fractionation

The bulk oil (2.13 g) was first chromatographed on a silica gel column (ICN 63-200 μm). Three fractions (F1, 1.142 g F2, 0.059 g and F3, 0.821 g) were eluted respectively with pentane (F1, F2) and diethyl oxide (F3). The fraction F3 was chromatographed on silica gel (ICN 63-200 μm) and 5 fractions (F3.1–F3.5 108, 35, 80, 61 and 130 mg, respectively) were eluted with a mixture of solvents (pentane/diethyl oxide) of increasing polarity. Final purification of isolyratols (16 mg) present in fraction F3.3 was achieved by chromatography on silica gel and exclusion size chromatography on sephadex LH-20 (elution with CH_2Cl_2).

3.3. Analytical GC

GC analysis was carried out using a Perkin–Elmer Autosystem apparatus equipped with FID and two fused-silica capillary columns (50 m × 0.22 mm i.d., film thickness 0.25 μm), BP-1 (polydimethyl siloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60 °C to 220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min; injector temperature: 250 °C; detector temperature: 250 °C; carrier gas:helium (1 ml/min); split:1/60. The relative proportions of the essential oil constituents were expressed as percentage obtained by peak area normalization. Retention indices (RI) were determined relative to the retention times of a series of *n*-alkanes with linear interpolation using the ''Target Compounds'' software from Perkin–Elmer.

3.4. GC–MS analysis

Samples were analysed with a Perkin–Elmer Turbo-Mass detector, directly coupled to a Perkin–Elmer Autosystem XL equipped with fused-silica capillary columns (60 m × 0.22 mm i.d., film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane). Ion source temperature: 150 °C; energy ionization: 70 eV; electron ionisation mass spectra were acquired oven the mass range 35–350 Da. Other GC conditions were the same as described under GC except split: 1/80.

3.5. NMR analysis

NMR spectra (^1H, ^{13}C, DEPT, COSY, HSQC and HMBC) were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.13 MHz for ^{13}C NMR, equipped with a 5 mm probe, in deuterochloroform, with all shifts referred to internal tetramethylsilane (TMS). ^{13}C NMR spectra were recorded with the following parameters: pulse width = 4μs (flip angle 45°); acquisition time = 2.7 s for 128 K data table with a spectral width of 25,000 Hz (250 ppm); CPD mode decoupling; digital resolution = 0.183 Hz/pt. The number of accumulated scans was 5000 for each sample (around 40 mg of the oil in 0.5 ml of $CDCl_3$).

3.6. Identification of components

Identification of the individual components was based: (i) on comparison of their GC retention indices (RI) on apolar and polar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation ('Target Compounds' software of Perkin–Elmer), with those of authentic compounds or the literature data, (ii) on computer matching with a laboratory made mass spectral library and commercial libraries (McLafferty and Stauffer, 1994; NIST, 1999), and comparison of spectra with the literature data (McLafferty and Stauffer, 1988; Joulain and König, 1998; Adams, 1995), (iii) on comparison of the signals in the ^{13}C NMR spectra of all the fractions of chromatography with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software (Tomi et al., 1995).

Otherwise, a few compounds were identified using the literature data:

- Yomogi alcohol **13**, and artemisia alcohol **26**. They were suggested by the computerized mass spectra libraries and confirmed by comparison of their ^{13}C NMR chemical shifts with the literature data (Ferreira et al., 1998).
- Lyratol **30** and lyratyl acetate **38** were identified by comparison of MS fragmentation patterns and ^{13}C NMR chemical shifts with the literature data (Näf-Muller et al., 1981; Ferreira et al., 1998).
- Two sesquiterpene aldehydes 3,9-dimethyl-6-isopropyl-2*E* and *Z*,7*E*,9-decatrienal **49** and **50**, which possess the sesquilavandulane skeleton, were identified by comparison of their MS and ^{13}C NMR data with those of reference compounds isolated and structurally characterized by us in a previous work (Ferrari et al., 2005).

3.7. Oxidation of lyratol

Lyratol (60 mg, 0.39 mmol), $CHCl_3$ (5 ml) and MnO_2(3 × 0.39 mmol) were stirred over night at room temperature. The reaction mixture was filtered. The organic phase was dried on $MgSO_4$ and concentrated in vacuum to yield lyratal **29** (56 mg, 0.31 mmol, 93% yield). ^1H NMR: δ 9.46 (1H, *s*, H-1), 6.46 (1H, *dq*, $J = 9.6$, 1.3 Hz, H-3), 5.85 (1H, *ddd*, $J = 17.2$, 10.2, 6.9 Hz, H-8), 5.15 (1H, *dt*, $J = 17.2$, 1.3 Hz, H-9a), 5.10 (1H, *dt*, $J = 10.2$, 1.3 Hz, H-9b), 4.89 (1H, *m*, $J = 1.3$ Hz, H-6), 4.84 (1H, *m*, H-6), 3.87 (1H, *dd*, $J = 9.6$, 6.9 Hz, H-4), 1.79 (3H, *d*,

$J = 1.3$ Hz, H-7) and 1.76 (3H, *m*, H-10) (in agreement with Bohlmann and Fritz, 1979), ^{13}C NMR data: 195.02 (C-1), 153.08 (C-3), 144.57 (C-5), 139.62 (C-2), 137.07 (C-8), 116.41 (C-9), 112.30 (C-6), 50.15 (C-4), 21.10 (C-10), 9.34 (C-7).

3.8. Oxidation of isolyratol and epi-isolyratol

A mixture of isolyratol **25** and *epi*-isolyratol **23** (15 mg, 0.1 mmol) dissolved in 2 ml of $CHCl_3$ was added to a suspension of pyridinium chlorochromate (43.5 mg, 0.2 mmol). The mixture was first stirred at 0 °C for 3 h and then at room temperature for 3 h. The reaction mixture was filtered and the solvent was removed under reduced pressure to yield isolyratone **16** (13.5 mg). The ketone **16** was submitted to 1D and 2D NMR analysis without further purification because of its rapid degradation (^{1}H NMR and ^{13}C NMR data, see Table 3) EIMS 70 eV, *m/z* (rel. int.): 135(5), 122(5), 107(8), 94(10), 81(8), 79(13), 77(7), 70(7), 69(90), 53(15), 42(8), 41(100), 39(58), 38(5).

3.9. Esterification of lyratol

3.9.1. Lyratyl butyrate **40**

To a solution of lyratol (80 mg, 0.53 mmol) in 30 ml CH_2Cl_2 and Et_3N (101 mg, 1 mmol) cooled to 0 °C, a solution of butyryl chloride (106 mg, 1 mmol) in CH_2Cl_2 (30 ml) was added drop wise. The mixture was stirred until room temperature and then refluxed during 3 h. The mixture was poured into 200 ml of cold water. After decantation, the organic phase was separated, washed twice with water, dried on Na_2SO_4 and concentrated in vacuum. The crude ester was purified by CC on silica gel. Yield = 32% (not optimised). EIMS 70 eV, *m/z* (rel. int.): 134(20), 121(12), 119(85), 106(17), 105(21), 93(28), 92(15), 91(35), 79(27), 77(22), 71(92), 55(15), 53(15), 43(100), 41(47), 39(21). ^{1}H NMRδ 5.80 (1H, *ddd*, $J = 17.9$, 9.6, 6.7 Hz, H-8), 5.46 (1H, br *d*, $J = 8.8$ Hz, H-3), 5.05 (1H, *dt*, $J = 9.6$, 1.5 Hz, H-9a), 5.03 (1H, *dt*, $J = 16.2$, 1.5 Hz, H-9b), 4.78 (2H, *m*, H-6), 4.50 (2H, br *d*, $J = 0.6$ Hz, H-1), 3.55 (1H, *dd*, $J = 8.8$, 6.7 Hz, H-4), 2.30 (2H, *t*, $J = 7.3$ Hz, H-12), 1.70 (3H, *m*, H-10), 1.67 (3 H, *m*, H-7), 1.65 (2H, *m*, H-13), 0.95 (3H, *t*, $J = 7.4$ Hz, H-14). ^{13}C NMR: 173.50 (C-11), 146.60 (C-5), 139.15 (C-8), 131.39 (C-2), 128.87 (C-3), 114.80 (C-9), 110.84 (C-6), 69.57 (C-1), 49.21 (C-4), 36.26 (C-12), 20.99 (C-10), 18.51 (C-13), 14.12 (C-7), 13.69 (C-14).

3.9.2. Lyratyl isovalerate **44**

Ester **44** was prepared as above: lyratol (53 mg, 0.35 mmol), Et_3N (62 mg, 0.8 mmol), isovaleryl chloride (102 mg, 0.8 mmol). The crude ester was purified by CC on silica gel. EIMS 70 eV, *m/z* (rel. int.): 134(22), 121(13), 119(100), 105(21), 93(28), 92(15), 91(40), 85(75), 79(30), 77(22), 57(83), 55(15), 53(12), 43(21), 41(45), 39(20). ^{1}H NMR δ 5.80 (1H, *ddd*, $J = 17.8$, 9.6, 7.0 Hz, H-8), 5.47 (1H, br *d*, $J = 8.2$ Hz, H-3), 5.05 (1H, *dt*, $J = 9.6$, 1.5 Hz, H-9a), 5.03 (1H, *dt*, $J = 17.8$, 1.5 Hz, H-9b), 4.78 (2H, *m*, H-6), 4.50 (2H, br *s*, H-1), 3.60 (1H, *dd*, $J = 8.2$, 7.0 Hz, H-4), 2.21 (2H, *d*, $J = 6.9$ Hz, H-12), 2.12 (1H, *m*, H-13), 1.71 (3H, br *s*, H-10), 1.68 (3H, br *d*, $J = 1.3$ Hz, H-7), 0.96 (6H, *d*, $J = 6.6$ Hz, H-14). ^{13}C NMR: 172.97 (C-11), 146.59 (C-5), 139.14 (C8), 131.37 (C-2), 128.97 (C-3), 114.80 (C-9), 110.83 (C-6), 69.55 (C-1), 49.21 (C-4), 43.49 (C-12), 25.75 (C-13), 22.42 (C-14), 20.98 (C-10), 14.15 (C-7).

3.9.3. epi-Isolyratol **23**

EIMS 70 eV, *m/z* (rel. int.): 83(10), 82(100), 81(14), 79(15), 77(8), 71(70), 67(97), 65(9), 55(12), 53(22), 43(57), 41(63), 39(45), 40(9).

3.9.4. Antibacterial activity

The test microorganisms included two Gram positive bacteria: *S. aureus* CIP 53.156 (ATCC 6538P) and *Listeria innocua* CIP 80.11 T (ATCC 33090) and four Gram negative bacteria: *Enterobacter aerogenes* CIP 60.86 T (ATCC 13048), *Escherichia coli* CIP 54.8 (ATCC 11775), *Pseudomonas aeruginosa* CIP A22, and *C. jejuni* NCTC 11168. In our study, *L. innocua* was used as a surrogate of *L. monocytogenes* (Girardin et al., 2005). All the strains were grown on Mueller-Hinton agar 2 with 5% of sheep blood (BioMérieux) at 30 °C or 37 °C. *C. jejuni* was grown on Mueller-Hinton agar 2 with 5% of sheep blood and Campylosel mixture (BioMérieux) under microaerophilic conditions by using the Genbox microaer system (BioMérieux).

The agar diffusion method (NCCLS, 1997) was used for the determination of antibacterial activities of the essential oil and the fractions. Briefly, a suspension of the tested microorganisms (2 ml for Petri dishes and 3 ml for 120×120 mm dishes of a suspension at 10^6 cells ml^{-1}) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 15 µl of the oil or fraction (diluted with DMSO 10%), placed on the inoculated plates and, after staying at room temperature for 1 h, they were incubated at 37 °C for *S. aureus* and *C. jejuni* and at 30 °C for *E. aerogenes*, *E. coli*, *P. aeruginosa* and *L. innocua*. Incubation time was 36 h for *C. jejuni* and 24 h for the other bacteria. Following incubation, zones of inhibition were measured (mm). Each test was performed in triplicate on at least three separate experiments. DMSO (Sigma) and antibiotics (penicillin G for *S. aureus*, amikacine for *C. jejuni*, rifampicine for *L. innocua* and ciprofloxacin for *E. coli* and *P. aeruginosa*, BioMérieux) were used as controls.

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