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Composition, Enantiomeric Distribution, and Antibacterial Activity of the Essential Oil of *Achillea ligustica* All. from Corsica

JEAN-JACQUES FILIPPI,*,†,‡ DON-ANTOINE LANFRANCHI,§ SOIZIC PRADO,# NICOLAS BALDOVINI,† AND UWE J. MEIERHENRICH†

LCMBA, UMR CNRS 6001, Université de Nice-Sophia Antipolis, Parc Valrose, F-06108 Nice Cedex 2, France; Bioanorganische Chemie 130, Universität Hohenheim, Garbenstrasse 30, D-70593 Stuttgart, Germany; Laboratoire de Stéréochimie, ECPM, 25 rue Becquerel, F-67008 Strasbourg Cedex 2, France; and Unité de Génétique Moléculaire Bactérienne, Unité de Chimie Organique, Institut Pasteur, 28 rue du Docteur Roux, F-75724 Paris Cedex 15, France

The essential oil of *Achillea ligustica* from Corsica was investigated by gas chromatography (GC) and gas chromatography−mass spectrometry (GC-MS). A total of 82 compounds representing 94.0% of the oil were tentatively identified. The main constituents were the camphane derivatives, representing >30% (camphor, 21.3%; borneol, 6.2%; bornyl acetate, 3.5%) of the whole oil, and santolina alcohol (19.3%). The enantiomeric distribution of 8 chiral constituents was determined by GC-MS using two enantioselective stationary phases (DIME-β-CD and Lipodex-E). Racemic santolina alcohol, required for optimization of the enantioselective GC conditions, was prepared by an original two-step synthesis from 2,5-dimethylhexa-2,4-diene. The whole essential oil was tested for its antibacterial activity against a wide range of bacteria using a paper disk method. The results show a promising activity against *Streptomyces* species.

KEYWORDS: ***Achillea ligustica*; essential oil composition; enantioselective analysis; antibacterial properties; santolina alcohol**

INTRODUCTION

Achillea ligustica is an herbaceous Asteraceae widespread in the Mediterranean area. In Corsica, this plant is found under the vernacular name "erba santa" (holy herb), which refers to its role in the Corsican traditions. Hence, it was used in cataplasms to relieve sprains and insect bites and had also a reputation for stopping hemorrhages (*1*). This is apparently a nontoxic plant as it has been traditionally added to cakes consumed on Good Friday.

Three studies have been reported on the composition of the essential oil of *A. ligustica*: Maffei et al. (*2*) found that samples prepared from plants collected in Italy were rich in artemisia ketone and 2,7-dimethyl-4,6-octadien-2-ol, whereas Tzakou et al. (*3*) showed that the hydrodistillation of *A. ligustica* harvested in Greece furnished essential oils containing linalool and 1,8-cineole as main constituents. Tuberoso et al. (*4*) recently reported the composition of samples of *A. ligustica* collected in different sites of Sardinia and showed that the essential oils were rich in santolina alcohol.

In the course of our studies on Mediterranean aromatic plants, we report here on the composition of Corsican *A. ligustica* essential oil, determined by means of gas chromatography (GC) and gas chromatography−mass spectrometry (GC-MS) techniques. Stereochemical configurations of the main components were measured by GC using chiral stationary phases, after oil fractionation. Additionally, the essential oil was also tested for its potential to inhibit *in vitro* the growth of various bacterial strains.

MATERIALS AND METHODS

Plant Material. The aerial parts of wild *A. ligustica* were collected at blooming stage at U Rugnicone, near Ajaccio (Corsica), in May 2004. A voucher specimen (voucher no. B-5071) was deposited in the herbarium of the Museum d'Histoire Naturelle de la ville de Nice, where the taxonomic identification of the plant material was confirmed.

Essential Oil Isolation. Fresh aerial parts (1000 g) were hydrodistilled for 5 h in a Clevenger-type apparatus. The essential oil was obtained as a pale blue liquid with a 0.4% yield (w/w).

GC and GC-MS Analyses. GC and GC-MS analyses on apolar columns were carried out using an Agilent 6890 N gas chromatograph apparatus equipped with a flame ionization detector (FID) and coupled to a quadrupole Agilent 5973 network mass selective detector working

* Address correspondence to this author at the Universität Hohenheim, Bioanorganische Chemie 130, D-70593 Stuttgart, Germany (e-mail jfilippi@uni-hohenheim.de or jfilippi@unice.fr).
† Université de Nice-Sophia Antipolis.
‡ Universität Hohenheim.
§ Laboratoire de Stéréochimie, ECPM.
# Institut Pasteur.

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in electron impact (EI) mode at 70 eV (scanning over 35−350 amu range). The gas chromatograph was equipped with two fused silica capillary columns HP-1 (PDMS, 50 m × 0.2 mm i.d., film thickness = 0.33 μm). The analytical parameters (identical for GC and GC-MS analyses unless specified) were the following: The carrier gas was helium at a flow rate of 1 mL/min. The oven temperature was programmed from 60 to 250 °C at 2 °C/min and held isothermal for 40 min. The injector (split mode, ratio 1/100) temperature was 250 °C. The FID temperature was set at 250 °C, and in the GC-MS analyses, the temperatures of the ion source and transfer line were 170 and 280 °C, respectively.

GC-MS analyses on polar column were performed with a Hewlett-Packard 5890 chromatograph coupled with a 5970A mass spectrometer of the same company (EI mode at 70 eV, mass range of 35−400 amu), using the following conditions: fused-silica capillary column, Agilent HP-20M (50 m × 0.2 mm i.d., film thickness = 0.1 μm); injection mode, split 1:30; oven temperature, programmed from 60 to 220 °C at 2 °C/min and then held isothermal for 30 min; carrier gas, helium (0.8 mL/min); injector and transfer line temperatures, 220 and 230 °C, respectively.

The constituents of the essential oil and its fractions were identified by comparison of their mass spectral pattern and retention indices (RI) with those of pure compounds registered in commercial libraries and literature data (*5*−*9*) or a laboratory-made database built from authentic compounds.

Enantioselective GC Analysis. Enantioselective GC analyses were performed using an Agilent 6890 gas chromatograph coupled with a 5973 mass selective detector (EI mode at 70 eV, mass range of 35−400 amu). Two different stationary phases were applied for this study. Hydrodex β-6-tBDM (25 m × 0.25 mm i.d.), Macherey-Nagel, Germany, was used with the following conditions: the oven temperature was held isothermal at 40 °C for 5 min and then programmed from 40 to 90 °C at 0.5 °C/min and then from 90 to 200 °C at 2 °C/min and held isothermal at 200 °C for 30 min. The carrier gas was helium, at a flow rate of 1.5 mL/min. The injector (split mode, 1/100) and the transfer line temperatures were 220 and 230 °C, respectively. In the case of β-pinene and sabinene, which presented coelution on the above-mentioned stationary phase, enantiomer separations were performed by using an enantioselective capillary Lipodex-E column (25 m × 0.25 mm i.d.; Macherey-Nagel, Germany) mounted on a HP 5890 gas chromatograph operated under the following conditions: the oven temperature was programmed from 40 to 180 °C at 1 °C/min. The carrier gas was nitrogen with a constant column head pressure of 11.5 psi. The injector (split, 1/100) and detector temperature was set at 200 °C.

NMR. NMR spectra were recorded on a Bruker DRX 500 spectrometer using deuterated chloroform as solvent. The chemical shift values are reported in parts per million with reference to TMS.

Chemicals. Standard compounds of definite enantiomeric purity were used to assess the enantiomeric ratios measured by GC-MS. Some of them were purchased from chemical supply companies: hex-3(*Z*)-en-1-ol (Acros, 928-96-1), benzaldehyde (Aldrich, B1334), (−)-α-pinene (Aldrich, 305715), (+)-camphene (Aldrich, C301), (+)-sabinene (Robertet, Grasse), (−)-β-pinene (Aldrich, 112089), β-myrcene (Aldrich, M100005), α-terpinene (Aldrich, 223182), *p*-cymene (Aldrich, C121452), limonene (Aldrich, 18,316-4), 1,8-cineole (Aldrich, C8-060-1), santolina alcohol (Fluka, 84500), γ-terpinene (Acros, 207501000), terpinolene (Fluka, 86484), linalool (Fluka, 62140), (+)-camphor (Aldrich, 857300), isoborneol (Aldrich, I13901), (−)-borneol (Acros, 464-45-9), (−)-terpinen-4-ol (Acros, 20126-76-5), α-terpineol (Acros, 301610250), myrtenol (Aldrich, W343900), eugenol (Aldrich, E51791), β-caryophyllene (Aldrich, W225207), caryophyllene oxide (Fluka, 22076). Chemicals for synthetic use including 2,5-dimethylhexa-2,4-diene, peracetic acid (40%), and vinylmagnesium bromide (1 M THF solution) were purchased from Sigma-Aldrich.

Essential Oil Fractionation. The essential oil (2.75 g) was fractionated by flash chromatography (FC) using pentane with increasing amounts of diethyl ether (0, 5, 10, 20, and 50%) as eluent. Five fractions were obtained, and the third fraction was further fractionated by column chromatography using 40−63 μm silica gel and 1% $AgNO_3$ doped silica gel, to provide 200 mg of santolina alcohol, pure enough for NMR identification.

Synthesis of Santolina Alcohol. In a 250 mL round-bottom flask placed in an ice bath was added dropwise 20 mmol of peracetic acid to a well-stirred mixture of 2,5-dimethylhexa-2,4-diene (2.16 g, 19.6 mmol) and Na_2CO_3 (8.34 g) in CH_2Cl_2 (50 mL). The reaction was stirred at room temperature until a negative KI−starch test was achieved. The reaction mixture was then washed with a saturated $NaHCO_3$ solution and brine. After drying, the solvent was evaporated under vacuum to provide 2.7 g of crude 2,2-dimethyl-3-(2-methylprop-1-enyl)oxirane as a pale yellow oil (the crude product contained mainly the expected epoxide; see Supporting Information Figure S4). This crude product was used directly in the next step: in a two-neck round-bottom flask placed in an ice bath and under magnetic stirring was added dropwise 20 mL of a 1 M THF solution of vinylmagnesium bromide to a well-stirred solution of crude 2,2-dimethyl-3-(2-methylprop-1-enyl)-oxirane (1.3 g; ≈8 mmol) in 5 mL of anhydrous diethyl ether under nitrogen atmosphere. The reaction was allowed to stand overnight at room temperature. The reaction mixture was then quenched with a saturated solution of ammonium chloride. The aqueous layer was extracted with diethyl ether, and the organic phases were collected, washed with brine, and dried over magnesium sulfate. The solvent was evaporated under vacuum to provide a crude pale yellow oil, which was purified by column chromatography on silica gel (40−63 μm) to yield 2,5-dimethyl-3-vinylhex-4-en-2-ol (santolina alcohol): ^{1}H NMR (Figures S1 and S2) δ 1.15 [s, 3H; C(OH)(C$\underline{H}_3$)$_2$], 1.17 [s, 3H; C(OH)-(C$\underline{H}_3$)$_2$], 1.65 [d, 3H, J = 1.4 Hz; CH=C(C$\underline{H}_3$)$_2$], 1.75 [d, 1H, J = 1.4 Hz; CH=C(C$\underline{H}_3$)$_2$], 2.98 (dd, 1H, J = 8.6, 9.5 Hz, H$_3$), 5.00−5.20 [m, 3H, C$\underline{H}$=C(C$\underline{H}_3$)$_2$, CH=C$\underline{H}_2$], 5.78 (ddd, 1H, J = 8.2, 9.6, 17.8 Hz, C$\underline{H}$=C$\underline{H}_2$); ^{13}C NMR δ 18.41, 26.37, 26.80, 27.12, 54.63, 72.75, 116.54, 122.74, 134.99, 138.07 [^{1}H and ^{13}C NMR spectra are in good agreement with those reported by Bertus et al. (*10*)]; EI-MS, *m*/*z* (%) 41 (18), 43 (26), 55 (12), **59** (**100**), 67 (11), 77 (8), 79 (16), 81 (81), 95 (8), 96 (67).

Bacterial Strains and Culture Conditions. References strains of *Escherichia coli* (ATCC 11775), *Corynebacterium jeikeium* (ATCC 43734), and *Nocardia asteroides* (ATCC 19247) were obtained from the Collection de l'Institut Pasteur, Paris, France. *Streptomyces coelicolor* (M15), *Streptomyces avidinii* (ATCC 31267), *Streptomyces albus*, *Pseudomonas aeruginosa* (13C3104), *Enterococcus faecalis* (14C1104), and *Staphylococcus aureus* (18C2204) strains were isolated from patients and kindly provided by Philippe Mazodier and Laurent Marsollier (Institut Pasteur). *N. asteroides* and *C. jeikeium* were cultivated for 48 h in brain heart infusion medium (BHI) at 30 and 37 °C, respectively. The other bacteria species were cultivated for 48 h in Mueller Huntington's medium (MH) at 37 and at 30 °C for *Streptomyces*.

Disk Diffusion Assay. The antibacterial activity of the essential oil was evaluated using the standardized filter paper disk (6 mm non-impregnated disk; antibiotica assay disks, grade 2668, Schleicher and Schuell) diffusion method according to the Kirby−Bauer method (*11*). Briefly, culture suspension of the tested microorganisms (≈10^6 CFU/mL) was spread on the solid medium plates (50 mL). Filter paper disks were impregnated with 10 μL of serial dilutions in dimethyl sulfoxide (DMSO, Sigma) of the essential oil and placed onto the solid medium plates. The diameter of inhibition was measured after 24 or 48 h of incubation at 30 or 37 °C. Ten microliters of ampicillin (1 mg/mL) (Sigma) and DMSO were used as positive and negative controls, respectively. All assays were performed in triplicate.

RESULTS AND DISCUSSION

GC-MS analyses were performed on the essential oil of *A. ligustica*, as well as on the five fractions obtained after FC of the whole oil, to further identify minor constituents and to separate some coeluting compounds. The chemical composition of the whole essential oil is reported in **Table 1**.

Among the main constituents, the camphane derivatives were the most abundant, representing 30.0% of the oil (camphor,

Table 1. Chemical Composition of *A. ligustica* Essential Oil

no.	component[a]	%[b]	RI HP1[c]	RI HP20M[c]	oil fraction	identification[d]	enantiomeric ratio[e]
1	hex-3(*Z*)-en-1-ol	0.1	833	1347	oil, F5	RI, MS, Std	
2	non-1-ene[f]	tr	886		F1	RI, MS	
3	santolinatriene[f]	1.3	902	1027	oil, F1	RI, MS	
4	artemisiatriene[f]	tr	918		F1	RI, MS	
5	tricyclene[f]	0.1	921		oil, F1	RI, MS	
6	α-thujene[f]	tr	923		oil, F1	RI, MS	
7	benzaldehyde	tr	927	1471	oil, F4	RI, MS, Std	
8	α-pinene	1.1	931	1002	oil, F1	RI, MS, Std	(1*S*,5*S*)-(–) 41:59 (1*R*,5*R*)-(+)[g]
9	camphene	2.5	944	1043	oil, F1	RI, MS, Std	(1*R*)-(+) 78:22 (1*S*)-(–)[g]
10	sabinene	0.3	965	1099	oil, F1	RI, MS, Std	(1*R*,5*R*)-(+) 89:11 (1*S*,5*S*)-(–)[h]
11	β-pinene	3.2	971	1083	oil, F1	RI, MS, Std	(1*S*,5*S*)-(–) 98:2 (1*R*,5*R*)-(+)[h]
12	β-myrcene	tr	981		F1	RI, MS, Std	
13	yomogi alcohol[f]	1.2	984	1377	oil, F3, F4	RI, MS	
14	isobutyl 2-methylbutyrate[f]	tr	988		F2	RI, MS	
15	isobutyl isovalerate[f]	tr	990		F2	RI, MS	
16	α-terpinene	0.3	1010	1153	oil, F1	RI, MS, Std	
17	*p*-cymene	1.0	1012	1240	oil, F1	RI, MS, Std	
18	limonene	tr	1019	1168	F1	RI, MS, Std	
19	1,8-cineole	1.0	1020	1176	oil, F2	RI, MS, Std	
20	santolina alcohol	19.3	1024	1375	oil, F3, F4	RI, MS, NMR, Std	(3*S*)-(+) 100: 0 (3*R*)-(–)[g]
21	artemisia ketone[f]	5.9	1044	1320	oil, F2	RI, MS	
22	γ-terpinene	0.9	1049	1219	oil, F1	RI, MS, Std	
23	*trans*-sabinene hydrate[f]	0.4	1053	1425	oil, F3	RI, MS	
24	artemisia alcohol[f]	0.5	1069	1476	oil, F3	RI, MS	
25	terpinolene	0.2	1079	1253	oil, F1	RI, MS, Std	
26	filifolone[f]	0.2	1082	1395	oil, F2	RI, MS	
27	linalool	1.4	1084	1515	oil, F3, F4	RI, MS, Std	
28	α-thujone[f]	1.9	1086	1377	oil, F2	RI, MS	
29	2-methylbutyl 2-methylbutyrate[f]	0.1	1090	1260	oil, F2	RI, MS	
30	*iso*-amyl isovalerate[f]	0.1	1093	1277	oil, F2	RI, MS	
31	β-thujone[f]	0.3	1097	1395	oil, F2	RI, MS	
32	chrysanthenone[f]	0.4	1099	1458	oil, F2	RI, MS	
33	4-acetyl-1-methylcyclohex-1-ene[f]	0.5	1105	1504	oil, F2	RI, MS	
34	*p*-menth-2-en-1-ol[f]	0.1	1108		oil, F5	RI, MS	
35	camphor	21.3	1123	1464	oil, F2, F3	RI, MS, Std	(1*S*,4*S*)-(–) 40:60 (1*R*,4*R*)-(+)[g]
36	*trans*-pinocarveol[f]	0.3	1124	1602	oil, F4	RI, MS	
37	camphene hydrate[f]	tr	1129	1545	F4	RI, MS	
38	pinocarvone[f]	0.1	1135	1514	oil, F2	RI, MS	
39	isoborneol	tr	1137		F4	RI, MS, Std	
40	*cis*-chrysanthenol[f]	0.2	1146	1642	oil, F4, F5	RI, MS	
41	lavandulol[f]	tr[i]	1149	1638	F4	RI, MS	
42	borneol	6.2[i]		1652	oil, F4	RI, MS, Std	(1*S*,2*R*,4*S*)-(–) 73:27 (1*R*,2*S*,4*R*)-(+)[g]
43	*p*-cymen-8-ol[f]	tr	1157	1796	oil, F5	RI, MS	
44	terpinen-4-ol	2.8	1162	1555	oil, F3, F4	RI, MS, Std	(4*S*)-(+) 56:44 (4*R*)-(–)[g]
45	artemisyl acetate[f]	0.2	1164	1331	oil, F2	RI, MS	
46	myrtenal[f]	0.2	1168	1570	oil, F2	RI, MS	
47	α-terpineol	0.6	1171	1650	oil, F4, F5	RI, MS, Std	
48	myrtenol	0.3	1177	1742	oil, F4	RI, MS, Std	
49	*trans*-carveol[f]	0.3	1196	1785	oil, F4	RI, MS	
50	*cis*-carveol[f]	0.1	1207		oil, F4	RI, MS	
51	carvone[f]	0.2	1212	1773	oil, F2	RI, MS	
52	*cis*-chrysanthenyl acetate[f]	0.9	1244	1533	oil, F2	RI, MS	
53	bornyl acetate[f]	3.5	1269	1540	oil, F2	RI, MS	
54	*trans*-sabinyl acetate[f]	0.2[j]	1272	1609	oil, F2	RI, MS	
55	lavandulyl acetate[f]	0.3[j]		1576	oil, F2	RI, MS	
56	unknown 1[j,k]	0.5	1285	1629	oil, F2		
57	*trans*-carvyl acetate[f]	0.4	1314	1695	oil, F2	RI, MS	
58	eugenol	0.3	1326	2100	oil, F4	RI, MS, Std	
59	*cis*-carvyl acetate[f]	0.3	1340	1727	oil, F2	RI, MS	
60	benzyl isovalerate[f]	0.1	1362	1844	oil, F2	RI, MS	
61	α-copaene[f]	0.1	1372	1459	oil, F1	RI, MS	
62	β-caryophyllene	0.3	1413	1555	oil, F1	RI, MS, Std	
63	α-humulene[f]	tr[i]	1442	1622	F1	RI, MS	
64	β-farnesene[f]	0.1[j]	1444	1641	oil, F1	RI, MS	
65	alloaromadendrene[f]	0.2[j]	1446	1559	oil, F1	RI, MS	
66	γ-muurolene[f]	0.1[j]	1465	1648	F1	RI, MS	
67	*ar*-curcumene[f]	0.2		1738	oil, F1	RI, MS	
68	germacrene-D[f]	3.1	1473	1667	oil, F1	RI, MS	
69	bicyclogermacrene[f]	0.2[j]	1488	1687	oil, F1	RI, MS	
70	ledene[f]	0.1[j]		1653	F1	RI, MS	
71	α-muurolene[f]	0.1	1490	1749	oil, F1	RI, MS	
72	δ-cadinene[f]	0.2[j]	1508	1718	oil, F1	RI, MS	
73	unknown 2[j,l]	0.1[j]		1770	oil, F2		
74	α-calacorene[f]	0.3	1524	1904	oil, F1	RI, MS	
75	palustrol[f]	0.2	1555	1870	oil, F2	RI, MS	

Table 1 (Continued)

no.	component[a]	%[b]	RI HP1[c]	RI HP20M[c]	oil fraction	identification[d]	enantiomeric ratio[e]
76	spathulenol[f]	0.1	1557	2061	oil, F4	RI, MS	
77	caryophyllene oxide	0.3	1563	1917	oil, F2	RI, MS, Std	
78	viridiflorol[f]	3.2	1576	2026	oil, F3, F4	RI, MS	
79	ledol[f]	0.3	1586	1968	oil, F4	RI, MS	
80	zingiberenol[f]	0.2	1593	2062	oil,F4	RI, MS	
81	unknown 3[j,m]	0.6	1601	2096	oil, F3, F4		
82	1-*epi*-cubenol[f]	0.8	1608	2006	oil, F2, F3	RI, MS	
83	β-eudesmol[f]	0.4	1627	2162	oil, F5	RI, MS	
84	cadalene[f]	tr	1645	2157	F1	RI, MS	
85	α-bisabolol[f]	0.4	1662	2168	oil, F4	RI, MS	
	identified compounds	82	94.0				
	monoterpene hydrocarbons	14	10.9				
	oxygenated monoterpenes	35	71.0				
	sesquiterpene hydrocarbons	14	5.1				
	oxygenated sesquiterpenes	9	5.9				
	others	10	1.2				

[a] Compounds are listed in order of their elution from an HP1 column. [b] FID percentage. tr = trace (<0.1%), trace levels of unidentified compounds are not mentioned. [c] RI= retention indices as determined on HP1 and HP20M columns using the homologous series of C_{5-26} *n*-alkanes. [d] Method of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; Std, by injection of an authentic sample (present in our laboratory-made database); NMR, after isolation of the compound by column chromatography and spectral analysis. [e] Determined by enantioselective gas chromatography (see Materials and Methods for conditions). [f] Compound tentatively identified according to its mass spectrum and by comparison of its retention indices with those of the literature. [g] Determined on heptakis(2,3-*O*,*O*-dimethyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (Hydrodex β-6TBDM). [h] Determined on octakis(3-*O*-butyryl-2,6-*O*,*O*-dipentyl)-γ-cyclodextrin (Lipodex E; Figure S3). [i] Percentage determined after analysis on HP20M column. [j] MS spectra of unknown compounds (see Supporting Information, Figure S5, for full spectra). [k] Unknown 1, *m*/*z* (%): 41 (28.7), 43 (100), 67 (10.5), 69 (23.9), 79 (15.4), 81 (17.6), 91 (17.6), 109 (95.4), 134 (33.3), 151 (27.3). [l] Unknown 2, *m*/*z* (%): 41 (51.0), 43 (91.2), 81 (65.0), 91 (75.1), 93 (48.7), 105 (100), 106 (71.5), 119 (45.5), 147 (45.6), 220 (96.2). [m] Unknown 3, *m*/*z* (%): 41 (46.9), 43 (47.2), 79 (44.2), 91 (54.2), 93 (37.4), 97 (52.2), 105 (41.2), 109 (39.1), 159 (100), 177 (36.3), 220 (7.3).

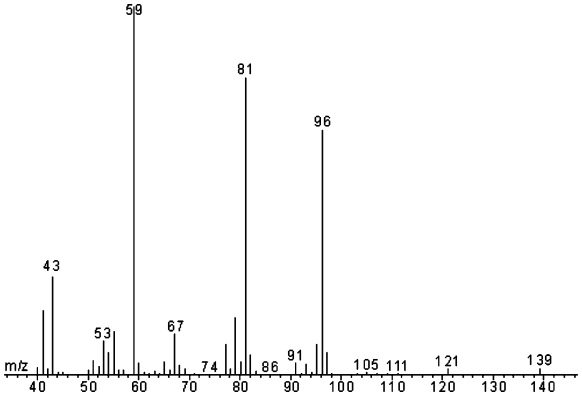

Figure 1. EI-MS spectrum of santolina alcohol.

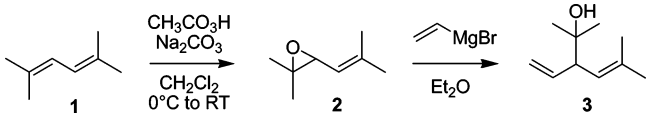

Figure 2. Synthesis of *rac*-santolina alcohol from 2,5-dimethylhexa-2,4-diene.

21.3%; borneol, 6.2%; bornyl acetate, 3.5%). According to the commercial spectral libraries and the literature (*7*, *12*), the second most abundant compound (RI 1024/1375) showed a mass spectrum identical with that of 2,7-dimethyl-4(*E*),6-octadien-2-ol, previously reported by Maffei et al. in the essential oil of northern Italian *A. ligustica* (*2*). However, as the retention index of this compound was not present in our databases, we could not confirm its identification by this crucial parameter. Then, we isolated this product from the oil by a multistep fractionation involving the use of $AgNO_3$-impregnated silica gel, and thereafter we could identify it undoubtedly as santolina alcohol by means of ^1H and ^{13}C NMR experiments and comparison with a commercial sample. The confusion between the mass spectral data of santolina alcohol (**Figure 1**) and 2,7-dimethyl-4(*E*),6-octadien-2-ol was pointed out by Näf-Müller et al. in 1981 (*13*).

In agreement with the literature (*2*, *4*), the sesquiterpenic content of the oil was mostly represented by germacrene-D (3.1%) and viridiflorol (3.2%). However, neither chamazulene nor guaiazulene was detected despite the specific blue color of the hydrocarbon FC fraction. Considering the high content and the variety of proazulenic lactones in *Achillea* species (*14*, *15*), other hitherto unknown azulenes can reasonably be present in the oil. As a result, *A. ligustica* from Corsica can be regarded as a potential new chemotype, completely different from the samples harvested in Greece (*3*), albeit closer to the northern Italian and Sardinian oils (*2*, *4*).

To determine stereochemical information on the main chiral constituents of the essential oil, enantioselective analyses were carried out on the five FC fractions (**Table 1**). Enantiomer resolution was achieved by using octakis(2,6-di-*O*-pentyl-3-*O*-butyryl)-γ-cyclodextrin (Lipodex-E) and heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (DIME-β-CD; Hydrodex β-6tBDM), which was previously reported to resolve monoterpenoids efficiently (*16*). Although chiral multidimensional GC is the most powerful tool for the chiral analysis of essential oils (*17*), the monodimensional chiral GC study of the FC fractions of the total oil is an economical alternative. The validity of the results requires a careful check of the absence of coelutions within the split peaks used for enantiomeric ratio determination (Figure S3). This check is based on the control of the nature of the constituents of the fractions on both chiral and achiral columns, as well as on the detailed examination of the mass spectral pattern of each split peak.

For all studied chiral compounds, analysis of racemic and/or enantiomerically enriched standard samples allowed us to determine the optimal chromatographic conditions required for their resolution and to attribute each peak to its corresponding enantiomer. As the commercially available santolina alcohol was enantiomerically pure, we had to prepare a racemic sample of this compound to determine the chromatographic conditions required for its chiral analysis. Racemic santolina alcohol **3** was then synthesized according to an original two-step procedure

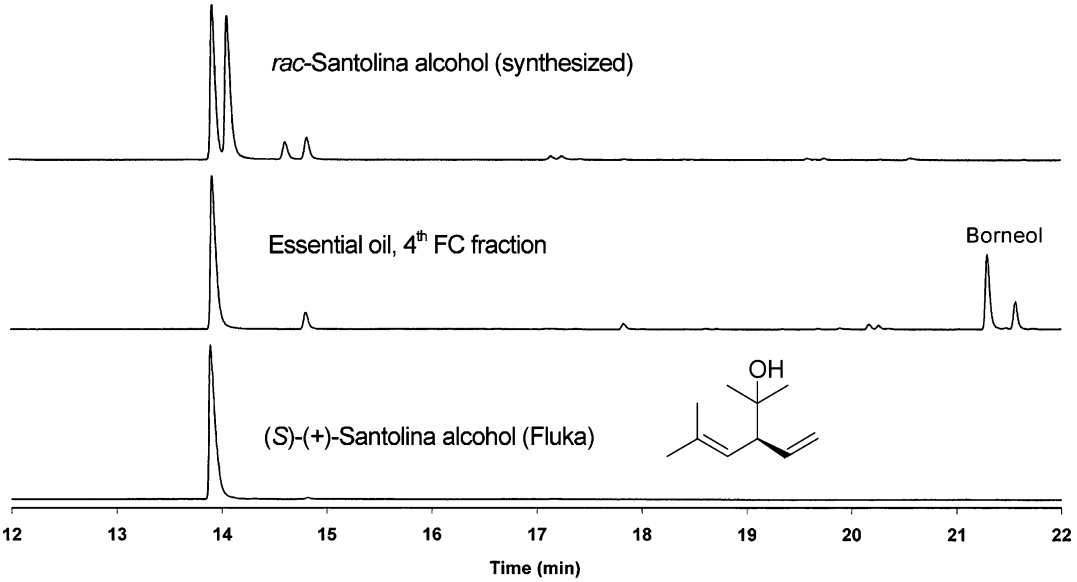

Figure 3. Enantiomer separation of santolina alcohol on heptakis(2,3-*O*,*O*-dimethyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin (Hydrodex β-6tBDM; see Materials and Methods).

Table 2. Antimicrobial Activity of *A. ligustica* Essential Oil As Determined by the Disk Diffusion Assay[a]

microorganism	property	oil concentration 100%	oil concentration 10%	oil concentration 1%	ampicillin 10 μg/mL
S. albus		20	17	9	07
S. avidinii		17	12	0	08
S. coelicolor		18	11	0	08
E. faecalis	Gram-positive	8	0	0	29
S. aureus	Gram-positive	9	7	6.5	15
P. aeruginosa	Gram-negative	9	7	0	0
E. coli	Gram-negative	8	7	0	21
N. asteroides		11.5	8	0	nd
C. jeikeium		17	1	0	20

[a] Values represent diameter of inhibition zone (mm) at indicated dilution (90/10) (EO/DMSO). No inhibition was observed for any of the tested strains with pure DMSO.

from commercial 2,5-dimethylhexa-2,4-diene **1**. This diene was epoxidized as described by Crandall et al. (*18*), and the crude oxirane **2** thus obtained (Figure S4) was used in the second step without purification, to be opened by vinylmagnesium bromide (**Figure 2**). *rac*-Santolina alcohol **3** was used to identify chromatographic conditions suitable for its enantiomeric resolution, and proved that the santolina alcohol contained in the *A. ligustica* essential oil was the pure dextrogyre form (**Figure 3**).

The antibacterial activity of the essential oil of *A. ligustica* was evaluated against Gram-positive (*E. faecalis*, *S. aureus*, *N. asteroides*, *C. jeikeium*), Gram-negative (*P. aeruginosa*, *E. coli*), and *Streptomyces* (*S. albus*, *S. coelicolor*, *S. avidinii*) bacteria. Antibacterial activity was determined by measuring the zone of growth inhibition of serial dilutions of the essential oil. Ampicillin was employed as positive control. The essential oil was found to have a significant antimicrobial activity against all of the tested bacteria. These results are in agreement with the data reported by Tuberoso et al. (*4*) except for the growth inhibition of *P. aeruginosa*. This variation could be explained by the different geographical origins of the samples. However, this activity was less potent than that of ampicillin except on *Streptomyces* strains. As shown in **Table 2**, the crude extract of essential oil and its 10^{-1} diluted solution possess a modest activity against Gram-positive and Gram-negative bacteria strains compared to control. No activity was observed at a dilution of 10^{-2} for these strains. Nevertheless, on *Streptomyces*, the diameter of inhibition is $\approx$17 mm, which is more than that for ampicillin (8 mm).

The antibacterial activity measured for the essential oil could be attributed to the major compounds, mainly camphor (21.3%) and santolina alcohol (19.3%). Indeed, the antimicrobial activity of various essential oils has been attributed to camphor (*19*−*21*). However, Yasphe et al. (*22*) measured the capacity of camphor to inhibit the growth of various strains, and no inhibition was observed against *E. coli* or *P. aeruginosa*. Hence, santolina alcohol and other minor constituents contributed probably to the antibacterial properties of this essential oil. Actually, antibacterial activities against *E. coli* and *P. aeruginosa* were reported for essential oils rich in santolina alcohol (*23*, *24*).

In conclusion, the essential oil of *A. ligustica* demonstrated an interesting selectivity as it has a modest antimicrobial activity against the tested Gram-positive and Gram-negative strains, but a very potent activity against *Streptomyces* bacteria, never described before. Further studies examining a large number of clinically isolated strains could be interesting to evaluate the therapeutic potential of this essential oil.

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Supporting Information Available: Figure S1, ^{1}H NMR spectrum santolina alcohol isolated from the oil; Figure S2, ^{13}C NMR spectrum (JMOD); Figure S3, enantiomer separation of sabinene and β-pinene (Lipodex E); Figure S4, ^{1}H NMR spectrum of 2,2-dimethyl-3-(2-methylprop-1-enyl)oxirane; Figure S5, EI-MS spectra of unknown compounds in *A. ligustica* essential oil. This material is available free of charge via the Internet at http://pubs.acs.org.

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