JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Seasonal and Phenological Variations of the Essential Oil from the Narrow Endemic Species *Artemisia molinieri* and Its Biological Activities

VÉRONIQUE MASOTTI,*,† FABIEN JUTEAU,† JEAN MARIE BESSIÈRE,§ AND JOSETTE VIANO†

Laboratoire Dynamique et Ressources du Végétal, E.A. 2202-Biodiversité, Université de Provence, UFR DENTES et SVTE, case 17, 3 place Victor Hugo, F-13331 Marseille Cedex 3, France, and Ecole Nationale Supérieure de Chimie de Montpellier, 8 rue Ecole Normale, F-34296 Montpellier, France

The volatile components of the aerial parts of *Artemisia molinieri*, an endemic wormwood of southern France, were analyzed by GC and GC-MS. Among the 69 compounds identified, major components were ascaridole (19−76%), α-terpinene (traces−36%), *p*-cymene (1−17%), 1,8-cineole (0.3−8%), and germacrene D (0.6−15%). Quantitative variations have been characterized following the season, the phenological cycle, and the aging of the plants. Bioassays have been performed on a sample of essential oil, which has shown a strong inhibition of the growth of both tested yeasts (*Candida albicans* and *Saccharomyces cerevisiae* var. *chevalieri*) and minor activity on both tested Gram-negative bacteria (*Escherichia coli* and *Enterococcus hirae*). The oils have shown interesting antioxidant activities on the basis of α-tocopherol as reference compound, up to 400−1200%.

KEYWORDS: ***Artemisia molinieri*; Asteraceae; essential oils; chemical composition; ascaridole; antibacterial and antifungal activities; antioxidant; chemiluminescence; GC; GC-MS**

INTRODUCTION

The genus *Artemisia* is one of the largest in the Asteraceae family, consisting of more than 800 species that are widespread all over the world. In this genus, some species are consumed as spices (tarragon) or alcoholic drinks (black and common wormwoods), and many of them have been used since ancient times as folk remedies and credited with a long list of medicinal uses, including antimalarial, antiviral, antitumor, spasmolytic, and others (*1*). Most of these interests can be related to the high amounts of volatile terpenic compounds that can be found in the essential oil, giving to the wormwoods their aromatic and medicinal properties (*2*−*4*). *Artemisia molinieri* [discovered by Quézel et al. (*5*)] is a restricted endemic species, located on two temporary freshwater marshes in southeastern France. This species has been registered in the Red Book of French flora as a plant threatened by extinction (*6*) and subjected to regional protection since 1994 in France. Both marshes are included in the LIFE international program and belong to the NATURA 2000 European network. As for many endangered species, it is important to determine highly valuable components in *A. molinieri* to assess protection status.

Previous analyses on *A. molinieri* have utilized diethyl ether extracts, which contain mainly ascaridole and two bisabolol oxide derivative (*7*). Some of the major compounds of its essential oil have been identified as 1,4-cineole and *p*-cymene (*8*) or α-terpinene, ascaridole, and *p*-cymene (*9*). The only exhaustive study of the essential oil of *A. molinieri* has been published by Carnat et al. (*10*), but these analyses deal with only one population, at flowering stage. These authors mentioned ascaridole, *p*-cymene, α-terpinene, 1,8-cineole, and germacrene D as major compounds.

A. molinieri antimicrobial activity has been tested on the flavonoid content of this species (*11*). These authors have reported antifungal activity equivalent to that of nystatine against *Candida albicans*.

It is important to note that the antioxidant activity of mugworts has not often been studied: the methanolic extract of *Artemisia maritima* has shown a weak antioxidant effect (*12*), but chlorogenic acid of *Artemisia iwayamogi* has shown the same activity as ascorbic acid (*1*), and the antioxidant power of *Artemisia judaica* essential oil was comparable to that of butylated hydroxytoluene (BHT).

MATERIALS AND METHODS

Plant Material. Aerial parts of *A. molinieri* Quézel, Barbero et R. Loisel, were harvested in southeastern France (departement of Var) in both known populations, near Besse-sur-Issole (Lac Gavoti) and near Flassans-sur-Issole (Lac Redon), at two different stages of development in 1999 (buds and flowering) and at four different stages of development in 2000 (vegetative, buds, flowering, and seeding). The aerial parts were harvested early in the morning, from numerous representative plants, randomly chosen. Plant material was taken immediately to the

* Corresponding author [telephone (33) 4 91 10 62 61; fax (33) 4 91 10 62 58; e-mail address: vmasotti@up.univ-mrs.fr].
† Université de Provence.
§ Ecole Nationale Supérieure de Chimie de Montpellier.

10.1021/jf034621y CCC: $25.00
Published on Web 10/22/2003

Table 1. Qualitative and Quantitative Composition (Peak Area Percent) of Seasonally Harvested *A. molinieri*[a]

location:				Lac Gavoti							Lac Redon							
harvest date:				Aug 1999		May 2000	Aug 2000		Oct 2000		Aug 1999		May 2000	Aug 2000			Oct 2000	
physiological status:				B	F	V	V	B	V	S	B	F	V	V	B	F	V	S
yield (%):				0.8	0.7	0.9	0.8	0.9	0.5	0.5	0.9	0.8	0.7	0.5	0.9	1.3	0.6	0.6
sample:				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	compound	identification	RI	peak area %														
1	α-thujene	abc	928	–	–	0.1	–	–	–	–	–	–	tr	–	–	–	–	–
2	α-pinene	abc	935	0.1	–	0.2	–	–	–	–	–	–	–	–	–	–	–	–
3	α-fenchene	abc	947	0.2	–	0.5	–	–	–	–	–	–	0.4	–	–	–	–	–
4	sabinene	abc	972	0.2	tr	0.7	–	0.1	–	0.1	tr	–	0.7	0.1	0.1	–	–	–
5	1-octen-3-ol	abc	973	–	–	–	–	–	–	–	–	–	0.1	0.1	–	–	–	–
6	β-pinene	abc	975	tr	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–
7	myrcene	abc	990	0.1	–	0.1	–	0.1	–	–	–	–	0.2	–	–	–	–	–
8	dehydro-1,8-cineole	abc	990	tr	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–
9	α-terpinene	abc	1022	10.6	0.5	34.2	0.7	6.9	tr	0.1	0.2	0.8	36.4	0.6	6.3	0.2	tr	tr
10	*p*-cymene	abc	1028	9.6	6.5	11.4	2.5	9.7	1.1	16.9	13.1	15.9	12.4	4.5	7.5	1.7	0.7	1.1
11	1,8-cineole	abc	1032	2.8	1.1	3.1	0.7	2.4	0.4	7.8	0.7	1.2	3.5	3.7	3.3	0.3	0.4	0.7
12	(*Z*)-β-ocimene	abc	1038	0.2	–	0.7	–	–	–	–	–	–	1.3	–	0.1	–	–	tr
13	(*E*)-β-ocimene	abc	1048	–	–	0.1	–	–	–	–	–	–	0.1	–	–	–	–	tr
14	γ-terpinene	abc	1059	0.6	0.2	1.3	0.2	0.3	0.1	0.2	0.3	0.1	1.3	0.2	0.5	0.1	0.1	0.2
15	NI		1065	0.1	–	–	–	–	–	–	tr	–	–	tr	0.1	tr	0.1	0.2
16	*cis*-sabinene hydrate	abc	1067	0.2	0.1	0.2	0.1	0.2	0.3	0.2	–	–	0.1	0.2	0.1	tr	0.1	0.1
17	terpinolene	abc	1088	0.1	–	0.2	tr	0.1	–	–	–	–	0.2	tr	0.1	–	–	tr
18	*p*-cymenene	abc	1088	0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.1	–	0.2	0.1	0.1	tr	–	0.2
19	*trans*-sabinene hydrate	abc	1098	0.2	0.2	0.4	0.1	0.2	0.3	0.2	0.1	–	0.2	0.2	0.1	0.1	0.4	0.2
20	2-methylbutyl 2-methylbutyrate	ab	1103	0.3	0.1	0.3	0.1	0.1	0.1	0.1	tr	–	0.3	0.1	0.1	0.1	–	0.2
21	amyl isovalerate	ab	1108	0.1	–	0.1	tr	tr	–	–	–	–	tr	–	–	–	–	–
22	*p*-mentha-1,3,8-triene	abc	1112	0.1	0.1	0.1	–	–	–	0.1	–	–	0.1	–	tr	–	–	–
23	*cis*-*p*-menth-2-enol	abc	1122	0.3	0.2	0.4	0.3	0.3	0.5	0.4	0.3	0.1	0.3	0.4	0.3	0.2	0.3	0.3
24	*p*-mentha-1,5,8-triene	a	1135	0.2	–	0.2	tr	0.1	–	0.1	0.1	tr	0.2	0.1	0.1	–	–	tr
25	*trans*-pinocarveol	abc	1136	0.1	–	–	0.1	0.1	tr	0.2	–	–	tr	tr	0.1	0.1	0.1	0.1
26	*trans*-*p*-menth-2-enol	abc	1141	0.4	0.1	0.3	0.3	0.3	0.4	0.4	0.1	0.1	0.2	0.2	0.2	0.1	0.3	0.3
27	NI		1148	0.1	0.1	0.2	–	–	–	–	–	–	tr	–	–	–	–	–
28	sabina ketone	abc	1153	0.1	–	–	0.1	0.1	–	0.1	–	–	–	–	tr	tr	0.1	tr
29	NI		1154	0.2	–	0.1	–	–	–	–	–	–	tr	0.1	tr	tr	–	tr
30	pinocarvone	abc	1161	0.2	–	–	–	tr	tr	0.1	–	–	–	–	tr	tr	0.1	tr
31	*trans*-β-terpineol	abc	1162	0.2	–	–	0.1	–	0.1	0.2	–	–	–	tr	0.1	tr	0.1	0.1
32	δ-terpineol	abc	1166	0.1	–	0.1	0.1	0.1	0.1	0.1	–	–	–	0.1	0.1	tr	0.1	0.1
33	rosefurane epoxide	abc	1167	–	–	0.1	–	–	–	–	–	–	tr	tr	tr	tr	–	0.1
34	NI		1168	0.2	–	0.2	0.1	0.2	0.2	0.2	–	–	0.3	0.1	0.1	tr	0.1	0.1
35	terpinen-4-ol	abc	1180	1.0	0.5	0.9	1.0	0.9	1.4	1.2	0.8	0.4	0.8	1.4	0.9	0.4	0.9	0.8
36	4-methylacetophenone	ab	1182	–	–	0.2	tr	tr	0.1	0.1	–	–	–	–	–	–	0.1	tr
37	cryptone	ab	1183	–	–	–	tr	tr	0.1	tr	–	–	–	tr	tr	–	–	–
38	*p*-cymen-8-ol	abc	1190	0.5	–	0.1	0.5	0.5	0.4	0.5	0.2	0.1	0.1	0.4	0.3	0.2	0.3	0.4
39	*cis*-piperitol	abc	1198	0.1	–	0.1	–	0.1	0.1	0.1	–	–	tr	0.2	tr	–	0.1	tr
40	*trans*-piperitol	abc	1208	tr	–	0.2	0.1	0.1	0.1	0.1	tr	–	0.1	0.1	tr	–	0.1	0.1
41	*cis*-isoascaridole	ab	1240	0.6	3.6	2.9	4.6	5.0	4.7	4.1	4.0	3.0	1.2	2.6	1.6	5.6	4.2	4.6
42	NI		1244	0.1	0.1	–	0.8	–	–	–	0.1	0.1	0.1	0.3	0.3	–	–	–
43	ascaridole	abc	1248	36.8	74.7	20.0	64.9	53.5	44.9	39.8	66.8	67.7	19.0	61.4	55.0	76.1	54.7	74.0
44	cumin aldehyde	abc	1252	0.2	tr	0.3	0.3	0.3	0.3	0.4	tr	tr	0.2	0.3	0.3	tr	0.2	0.2
45	NI		1253	–	–	0.1	0.2	0.2	–	–	–	–	0.1	0.1	–	–	–	–
46	*cis*-piperitone oxide	ab	1254	1.2	0.5	0.8	1.0	0.9	0.5	1.0	0.8	0.5	0.8	0.9	1.1	1.0	0.9	1.2
47	*trans*-piperitone oxide	ab	1258	0.6	0.7	0.5	0.7	0.8	0.9	1.1	0.6	0.3	0.2	0.7	0.9	0.3	0.7	0.9
48	*cis*-carvenone oxide*	a	1262	2.3	2.6	1.0	–	tr	7.6	7.5	3.4	2.3	1.2	3.2	4.5	1.8	6.4	5.0
49	*trans*-carvenone oxide*	a	1272	0.4	0.6	1.8	0.6	0.8	1.8	1.2	–	–	0.1	0.8	0.6	0.4	1.5	0.5
50	NI		1283	0.1	0.1	–	–	–	–	–	0.1	–	tr	0.2	0.2	0.2	0.3	0.2
51	NI		1285	–	–	–	–	–	–	–	tr	–	tr	0.2	0.2	0.1	0.2	0.1
52	thymol	abc	1286	0.6	1.7	0.3	5.5	0.6	1.2	0.6	1.3	1.2	0.2	0.6	0.5	0.2	0.7	0.5
53	NI		1290	0.2	0.2	–	0.3	0.2	0.3	0.4	0.2	0.3	0.2	0.4	0.4	0.2	0.3	0.3
54	*trans*-isoascaridole	ab	1295	2.6	1.5	1.1	1.6	2.0	1.4	1.1	1.7	1.2	0.9	2.2	2.3	3.0	1.6	2.6
55	carvacrol	abc	1296	1.1	0.7	0.5	1.0	1.0	1.6	0.8	0.9	0.7	0.5	1.2	1.1	1.4	1.1	0.7
56	cumin alcohol	abc	1348	0.2	–	–	0.1	0.1	tr	–	tr	–	0.1	0.1	0.1	–	–	–
57	eugenol	abc	1360	0.4	0.1	0.1	0.1	0.1	tr	–	0.1	0.1	0.2	0.1	0.1	0.1	–	0.1
58	NI		1368	tr	–	–	tr	0.1	0.1	–	tr	–	–	tr	–	–	0.1	–
59	α-copaene	abc	1382	0.3	0.1	tr	0.1	0.2	0.3	0.2	–	–	–	0.2	0.2	0.1	0.1	0.1
60	β-bourbonene	abc	1390	tr	–	–	tr	–	tr	tr	–	–	0.1	–	tr	–	–	0.1
61	(*Z*)-jasmone	abc	1399	0.4	0.1	0.2	0.1	tr	0.1	0.1	0.2	0.1	0.4	0.1	0.2	0.2	0.1	0.2
62	β-caryophyllene	abc	1425	0.2	–	tr	0.1	0.2	0.2	0.1	–	–	0.2	0.1	0.1	0.1	0.1	0.1
63	(*E*)-β-farnesene	abc	1460	0.4	–	0.2	0.2	0.1	–	–	–	–	0.2	0.1	0.1	tr	tr	–
64	dehydrosesquicineole	abc	1460	0.1	–	0.4	0.1	0.1	tr	tr	–	–	0.6	0.1	tr	–	–	–
65	linalyl 2-methylbutyrate	ab	1461	0.9	0.1	0.7	1.0	1.1	2.6	1.3	0.4	0.1	0.6	0.8	0.5	0.5	0.6	0.2
66	γ-curcumene	abc	1482	–	–	0.3	0.1	0.3	–	–	0.1	0.1	0.5	–	–	–	–	–
67	germacrene D	abc	1486	4.1	0.6	3.5	3.0	3.8	15.1	7.3	1.9	2.6	4.9	6.5	5.5	1.9	7.4	0.9
68	β-selinene	abc	1489	0.2	–	–	0.2	0.3	0.3	0.1	–	–	–	0.1	0.1	0.1	0.1	0.1

Table 1. (Continued)

location:			Lac Gavoti							Lac Redon							
harvest date:			Aug 1999		May 2000	Aug 2000		Oct 2000		Aug 1999		May 2000	Aug 2000			Oct 2000	
physiological status:			B	F	V	V	B	V	S	B	F	V	V	B	F	V	S
yield (%):			0.8	0.7	0.9	0.8	0.9	0.5	0.5	0.9	0.8	0.7	0.5	0.9	1.3	0.6	0.6
sample:			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	compound	identification	RI	peak area %													
69	isoamyl phenylacetate	a	1490	–	tr	0.3	0.2	–	0.2	0.1	0.1	tr	0.3	0.1	0.2	tr	0.2	tr
70	bicyclogermacrene	abc	1499	0.4	0.1	0.2	0.2	0.2	0.5	0.3	0.1	0.1	0.3	0.2	0.2	0.2	0.3	tr
71	linalyl valerate	ab	1510	0.3	–	tr	0.2	0.2	0.2	0.1	–	–	–	0.1	0.1	tr	tr	tr
72	δ-cadinene	abc	1524	0.1	–	tr	tr	tr	tr	tr	–	–	tr	–	tr	–	–	–
73	(*E*)-nerolidol	abc	1559	tr	–	–	–	–	–	–	–	–	–	0.1	0.1	–	–	–
74	NI		1573	0.2	–	tr	–	0.1	0.2	0.1	–	–	0.1	0.1	0.1	–	0.2	0.1
75	spathulenol	abc	1574	0.1	0.1	–	0.3	0.2	0.4	0.1	0.1	–	–	0.3	0.2	0.1	0.2	0.1
76	salvialenone	ab	1608	–	–	0.3	0.2	0.2	0.4	0.1	tr	–	–	tr	0.1	–	–	0.1
77	NI		1637	–	–	–	–	–	0.2	–	–	–	0.3	–	0.1	0.1	0.3	0.1
78	linalyl 3-methylhexanoate	ab	1654	0.2	–	tr	0.1	0.2	0.3	0.2	tr	–	0.1	0.1	0.1	0.1	0.1	–
79	α-bisabolol oxide B	abc	1656	2.5	0.1	0.6	1.1	0.7	1.3	0.6	0.2	0.1	0.5	0.7	0.4	0.4	1.9	0.7
80	α-bisabolol	abc	1681	0.6	0.1	0.4	0.3	0.3	0.6	–	0.1	0.1	0.3	–	0.1	0.2	0.1	0.1
81	NI		1730	0.3	0.1	tr	–	tr	0.1	–	0.1	–	–	–	–	–	tr	–
82	α-bisabolol oxide A	abc	1746	1.4	0.6	0.6	0.3	0.3	0.6	tr	0.2	0.1	0.4	0.1	0.3	0.4	0.1	0.1
83	NI		1765	0.3	0.1	–	–	tr	0.1	–	tr	–	–	–	–	–	0.1	–
84	NI		1806	0.1	0.1	–	0.2	0.1	0.2	0.3	0.1	–	–	0.3	0.1	0.1	0.9	0.1
85	NI		1832	4.7	0.4	2.9	1.5	1.3	2.2	0.6	0.3	0.2	2.8	1.1	0.8	0.8	3.3	0.5
86	NI		1847	1.0	0.3	0.1	0.2	0.2	0.3	0.2	0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.2
87	NI		1860	4.4	0.2	2.7	1.4	1.2	2.4	0.6	0.1	0.3	2.8	0.5	0.4	0.5	6.3	0.1
	total identified (%)		88.0	98.3	93.7	95.3	96.4	93.7	97.6		99.0	99.0	93.2	96.5	97.0	97.7	87.6	98.1

[a] RI, retention indices relative to C_8–C_{22} *n*-alkanes on DB5 column; V, vegetative plant; B, budding plant; F, flowering plant; S, seeding plant; 1, a = mass spectra, b = retention index, c = comparison with standard; tr, traces (<0.1%); *, tentative identification; NI, not identified (see **Table 2** for mass spectra data); –, not detected.

laboratory to be dried at ambient temperature, with ventilation. Drying time was ~72 h. Voucher specimens were deposited in the Herbarium of the University of Provence, France (MARS-2000.6).

Isolation of the Essential Oils. Dried material was powdered in a Tecator Cyclone mill (mesh width = 1 mm), and 100 g was immediately hydrodistillated in a Clevenger type apparatus for 2 h (*13*). The essential oils were pale yellow and liquid at ambient temperature, with a strong woody fragrance. The essential oil was stored at 4 °C in the dark, until the moment of analysis.

GC and GC-MS Analysis. Capillary gas chromatography was carried out using a Varian (model 3900GC) chromatographic system with a flame ionization detector (FID), equipped with a CP SIL 8CB fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness). Oven temperature was programmed from 50 to 220 °C at 3 °C/min, after an isothermal step at 50 °C for 2 min. The carrier gas was He, with a flow rate of 0.5 mL/min. Injector and detector were heated at 220 and 230 °C, respectively. The injection volume was 0.1 μL for each sample.

GC-MS analyses were carried out on a Hewlett-Packard (model 5790) capillary gas chromatograph quadrupole mass spectrometry system fitted with a DB5 fused silica capillary column (50 m × 0.2 mm, 0.25 μm film thickness). Chromatographic conditions were the same as mentioned above, and the mass spectrometer was operated at 70 eV.

Component identification was carried out by comparison with authentic reference compounds, spectrometric electronic libraries (Wiley 138, NBS 75K), published mass spectra (*14*), and retention indices (*15*). Quantitative analysis of each oil component (expressed in percent) was carried out by peak area normalization measurements.

Antimicrobial Activity. Bactericidal and fungicidal activities of *A. molinieri* essential oils were determined according to a liquid diffusion method (*16*), modified as described previously (*17*). The concentrations used were in the range of 0.78−100 μg mL^{-1}. Antibacterial and antifungal activities were determined in terms of growth inhibitory concentration for 50% of the microorganisms (GIC_{50}, mg mL^{-1}) and complete inhibition concentration (mg mL^{-1}) (CI). Standard antibiotics (penicillin G and nystatine) were used to control the sensitivity of the tested microorganisms. The antibacterial activity of the essential oil has been tested against the two Gram-positive bacteria *Staphylococcus aureus* (CIP 53154) and *Enterococcus hirae* (CIP 5855) and the Gram-negative bacteria *Escherichia coli* (CIP 54127); these strains were cultivated for 24 h at 37 °C on Mueller−Hinton medium. The antifungal activities have been tested against the yeasts *Candida albicans* (CIP 1180-70) and *Saccharomyces cerevisiae* var. *chevalieri* (ATCC 28383); the tested organisms were previously maintained for 24 h at 28 °C on Sabouraud medium. These bacterial strains are those recommended by the French Normalization Association (NFT 72-150) to define bactericidal activity of liquid antiseptics.

Antioxidant Activity. Antioxidant activity has been measured by chemiluminescence using a Yelen luminometer, as previously described by Mantle et al. (*18*). The reaction mixture (220 μL) contained 0.03 μM AAPH [2,2-azobis(2-amidinopropane) dihydrochloride], 50 μM luminol (luminescent Biostab reagent), and 20 μM of an appropriate dilution of the reference compound (α-tocopherol) or essential oil (*19*). The latter compounds were previously diluted in ethanol prior to a series of dilutions in distilled water. Chemiluminescence intensities of both blank (M1) and assay (M2) were monitored by integration over 1 min, and the percentage of inhibition (Inh%) was calculated using the following formula: Inh% = 100(1 − M2/M1). The results were expressed as the concentration of the test sample that shows 50% inhibition of α-tocopherol chemiluminescence (IC_{50}).

RESULTS AND DISCUSSION

Essential Oils. The identity, retention index, and percent composition of each oil of *A. molinieri* are presented in **Table 1**. **Figure 1** shows a typical gas chromatogram of vegetative plant oil. The yields are based on dry weight of each sample and ranged between 0.5 and 1.3% (**Table 1**). Among the 15 analyses, 69 compounds were identified, representing 87.6−99.0% of the oils, mainly terpenic components. Among the identified compounds were 15 monoterpene hydrocarbons (0.8−

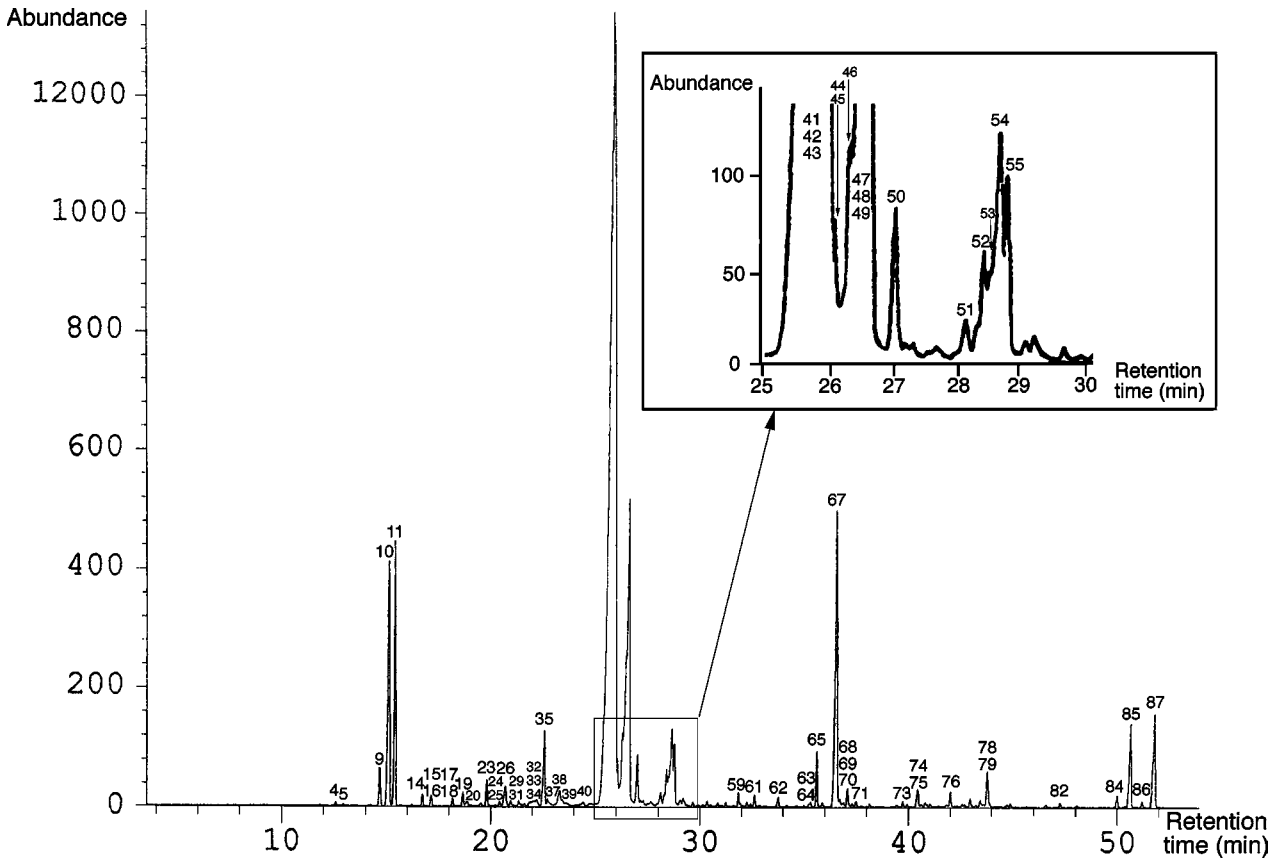

Figure 1. Typical gas chromatogram of volatile compounds of aerial parts of *A. molinieri* (sample 11).

Table 2. Mass Spectral Data of Unidentified Volatile Compounds of *A. molinieri*

NI	RI	MS, *m/z*
15	1065	43 (100), 99 (53), 71 (22), 41 (20), 93 (12), 136 (3)
27	1148	119 (100), 109 (95), 43 (50), 124 (47), 95 (43), 134 (28), 152 (24), 137 (18)
29	1154	123 (100), 43 (40), 81 (40), 166 (32), 95 (26), 108 (15)
34	1168	109 (100), 79 (14), 81 (10), 91 (10), 152 (9)
42	1244	43 (100), 99 (26), 141 (17), 41 (15), 69 (13), 71 (13)
45	1253	43 (100), 41 (43), 121 (15), 141 (10), 93 (9), 136 (5)
50	1283	135 (100), 150 (35), 91 (31), 43 (23), 115 (18), 109 (16)
51	1285	43 (100), 95 (80), 110 (78), 41 (50), 67 (41), 126 (18), 168 (11)
53	1290	135 (100), 150 (25), 91 (14), 115 (10), 107 (9)
58	1368	125 (10), 41 (50), 43 (47), 97 (42), 126 (19), 150 (8)
74	1573	41 (100), 96 (88), 81 (65), 43 (58), 123 (55), 95 (50), 67 (47), 122 (33), 177 (15), 159 (14), 149 (14)
77	1637	43 (100), 134 (56), 145 (48), 121 (43), 41 (40), 119 (37), 79 (27), 93 (25), 96 (23), 178 (7), 160 (4)
81	1730	43 (100), 143 (50), 71 (33), 125 (21), 91 (21), 119 (13), 134 (4)
83	1765	43 (100), 143 (56), 71 (36), 91 (27), 125 (25), 134 (8)
84	1806	43 (100), 132 (85), 119 (23), 91 (16), 158 (15), 201 (7), 219 (6), 261 (2)
85	1832	43 (100), 125 (26), 143 (24), 185 (16), 91 (12)
86	1847	43 (100), 125 (30), 143 (25), 185 (22)
87	1860	43 (100), 125 (22), 143 (21), 185 (18), 91 (11)

53.4%), 28 oxygenated monoterpenes (29.4−92.7%), 13 sesquiterpene hydrocarbons (0.9−19.6%), and 7 oxygenated sesquiterpenes (0.9−5.6%). The major compounds in the oils were ascaridole (19−76.1%), α-terpinene (traces−36.4%), *p*-cymene (0.7−16.9%), 1,8-cineole (0.3−7.8%), and germacrene D (0.6−15.1%). These major compounds have previously been identified by Carnat et al. (*10*), but some compounds that were present in most of the investigated oils were not noticed by these authors,

Table 3. Antibacterial Activity of *A. molinieri* Essential Oil (Vegetative Status, Sample 3)[a]

microorganism	essential oil	penicillin G	nystatine
E. coli	$GIC_{50} = 0.1$ $CI = 0.2$	$GIC_{50} = 0.03$ $CI = 0.05$	NT
S. aureus	$GIC_{50} = 0.1$ $CI = 0.2$	$GIC_{50} = 3 \times 10^{-4}$ $CI = 5 \times 10^{-4}$	NT
E. hirae	–	$GIC_{50} = 3 \times 10^{-4}$ $CI = 8 \times 10^{-4}$	NT
C. albicans	$GIC_{50} = 0.1$ $CI = 0.2$	NT	$GIC_{50} = 3 \times 10^{-3}$ $CI = 6 \times 10^{-3}$
S. cerevisiae var. *chevalieri*	$GIC_{50} = 1.25 \times 10^{-4}$ $CI = 5 \times 10^{-4}$	NT	$GIC_{50} = 3 \times 10^{-3}$ $CI = 6 \times 10^{-3}$

[a] GIC_{50} = growth inhibitory concentration for 50% of the microorganisms (mg/mL); CI = complete inhibition concentration (mg/mL); NT, not tested; –, no effect at tested concentrations.

that is, linalyl butyrate, bicyclogermacrene, *p*-cymenene, β-caryophyllene, α-copaene, spathulenol, *cis*-sabinene hydrate, and linalol. However, they were minor components. Most of the monoterpenic components were derivates of α-terpenyl intermediate (**Figure 2**). Sesquiterpenes were mainly represented by germacrene D, α-bisabolol, and α-bisabolol oxides A and B. Thus, and considering the mass spectral data of unidentified heavy compounds **81** and **83**−**87** (**Table 2**), we suggest that these compounds may be bisabolol derivatives. All of the essential oils contained only low levels of nonterpenic components. For example, eugenol, (*Z*)-jasmone, and isoamyl phenylacetate, when present, represented <1% of the oil.

On one hand, it seems that phenological status led to straight variations of the essential oil compositions. The higher rates of α-terpinene (∼35%) were found in vegetative stage harvested

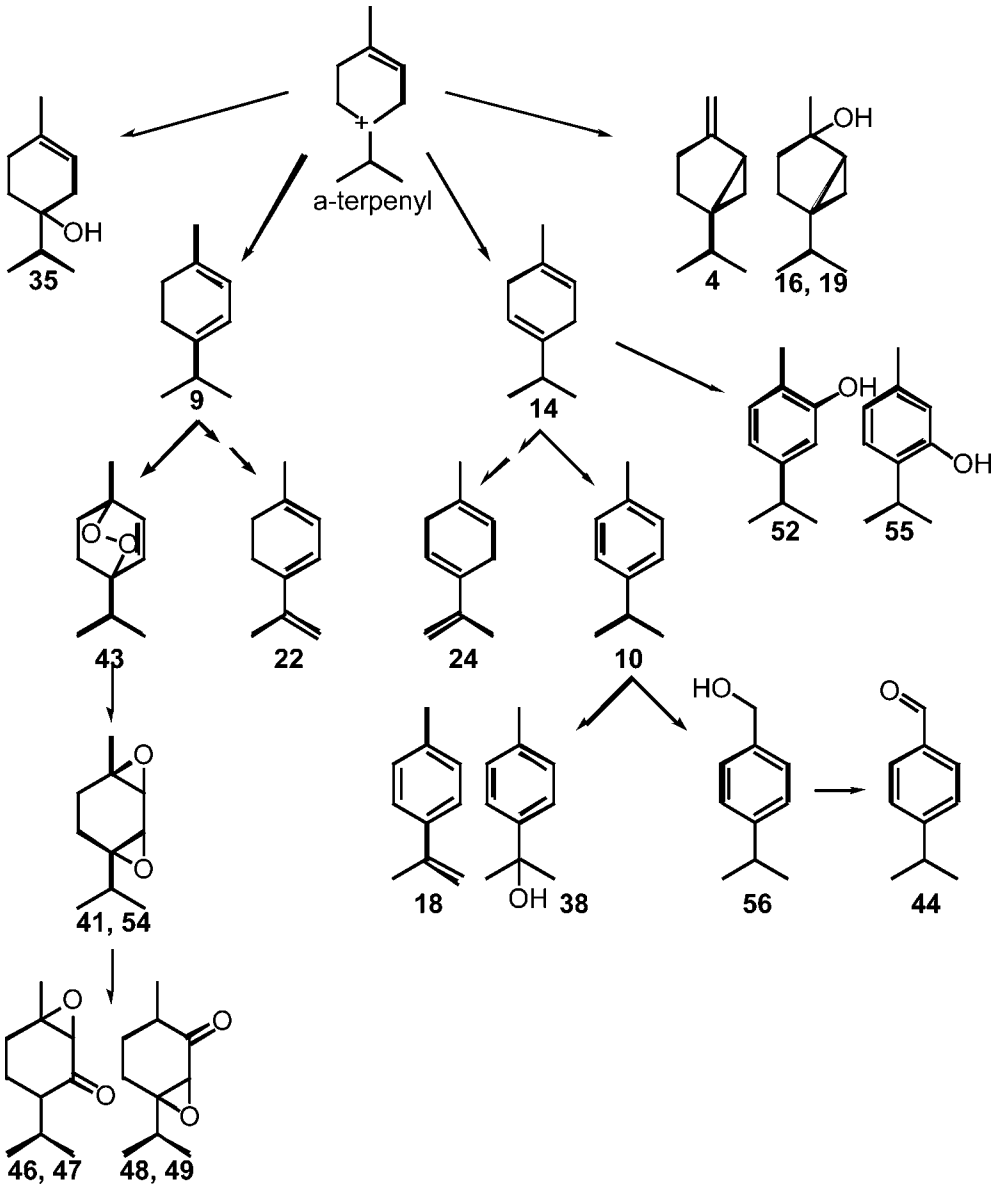

Figure 2. Biosynthetic pathway of the main constituents of *A. molinieri* essential oil.

in spring, in both marshes (samples 3 and 10); these oils were those that contain lower rates of ascaridole (~20%). During flowering, the proportion of ascaridole increased while that of α-terpinene decreased: at bud stage (samples 1, 5, and 12) the ascaridole ranged from 37 to 55%, whereas α-terpinene fell to 10%. At flowering or seeding stages, the proportion of α-terpinene decreased to <1%, while ascaridole attained 67−76% (samples 2, 9, 13, and 15). The correlation between these compounds may be due to the biosynthetic pathway of ascaridole: following Johnson and Croteau (*20*) results on another plant species (*Chenopodium ambrosioides*) α-terpinene lead to ascaridole with a single enzyme, an iodide peroxidase. Thus, it would be easy to conclude that phenological status can influence the regulation of the biosynthesis of essential oil, as it has already been involved in other *Artemisia* species [*A. absinthium* (*10*)].

On the other hand, great variations of the oil compositions were noticed for plants at the same phenological stage, vegetative, for example, harvested in spring (samples 3 and 10), in summer (samples 4 and 11), or in autumn (samples 6 and 14): α-terpinene decreased and ascaridole increased through the year. Moreover, essential oil compositions were similar between young shoots (sample 11) and older plants (samples 12 and 13) harvested at the same period. Thus, it seems that variations of α-terpinene and ascaridole were not correlated to physiological status, but rather by environmental conditions. This is why we have initiated research with plants cultivated in strictly controlled conditions in order to specify which factor (light, temperature, or water) has the major influence on *A. molinieri* secondary metabolism.

Antimicrobial Activities. The oil of vegetative aerial parts of *A. molinieri* (sample 3) showed mild activity against both bacteria *E. coli* and *S. aureus* and against the fungus *C. albicans* (**Table 3**). Previous studies have pointed out that undiluted oils with oxygenated-rich components have significant effects on a large variety of bacteria (*21*). However, our volatile extract contains <37% of oxygenated components, but a complete inhibition of fungal growth was observed against *S. cerevisiae* with only 50 μg mL^{-1} of *A. molinieri* oil, and antifungal effects are perceptible from 12.5 μg mL^{-1}. Biological activity of crude

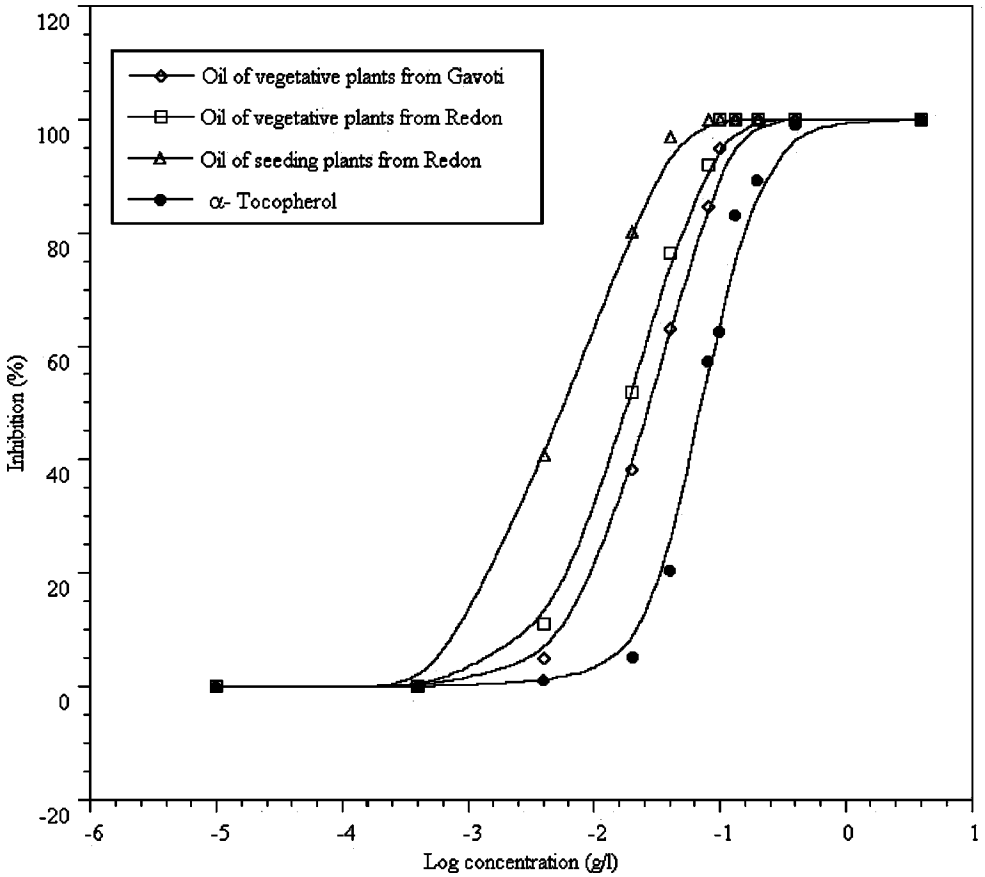

Figure 3. Antioxidant activities of the essential oil of the aerial parts of *A. molinieri* (samples 3, 10, and 15) plus α-tocopherol in chemiluminescence assay.

vegetable extract is uncommon at such concentrations. By the way, the third main components, representing 66% of the oil, are recognized as powerful antifungal components: α-terpinene (*22*), *p*-cymene (*23*), and ascaridole (*24*, *25*). Ascaridole is known to be the principal allelochem (with regard to α-terpinene and *p*-cymene) in *Chenopodium ambrosioides* (*26*) and to have an important impact on microbial growth (*27*).

Antioxidant Activities. Both essential oils of vegetative plants (Lac Gavoti, sample 3, and Lac Redon, sample 10) showed strong antioxidant activities, higher than that of the control, 250 and 380%, respectively (**Figure 3**). High antioxidant activity was expected because α-terpinene and *p*-cymene, representing almost 50% in each oil, are known as antioxidants similar to α-tocopherol (*21*). The other main compound, that is, ascaridole (∼20%), could explain such activity, but as far as we know, the antioxidant activity of pure ascaridole is unknown. Thus, we have investigated the antioxidant activity of sample 15, in which ascaridole attained 74% of the oil. The antioxidant activity of this sample was 12 times higher (1240%) than that of α-tocopherol. It seems that ascaridole is a powerful antioxidant.

In conclusion, we have established that the metabolism of the essential oil of a restricted endemic species of wormwood, *A. molinieri*, was much more related to environmental conditions than to physiological status (i.e., flowering and/or aging). Thus, the need for ex situ cultivation to study the mechanisms that lead to the biosynthetic pathway of secondary metabolites of high interest for pharmaceutical sponsors is clear.

ACKNOWLEDGMENT

We thank Dr. M. Dherbomez (SMAB, IUT La Rochelle, France) for helpful cooperation on antimicrobial activities and Prof. Dr. A. Lavagne (University of Provence, France) for assistance in the collection and identification of the plant.

LITERATURE CITED

(1) Tan, R. X.; Zheng, W. F.; Tang, H. Q. Biologically active substances from the genus *Artemisia*. *Planta Med*. **1998**, *64*, 295−302.

(2) Knobloch, K.; Pauli, A.; Iberl, B.; Weigand, H.; Weis, N. Antibacterial and antifungal properties of essential oil components. *J. Essent. Oil Res*. **1989**, *1*, 119−128.

(3) Juteau, F.; Bessière, J. M.; Masotti, V.; Viano, J. Compositional characteristics of the essential oil of *Artemisia campestris* var. *glutinosa*. *Biochem. System. Ecol*. **2002**, *30*, 1065−1070.

(4) Juteau, F.; Masotti, V.; Bessiere, J. M.; Dherbomez, M.; Viano, J. Antibacterial and antioxidant activities of *Artemisia annua* essential oil. *Fitoterapia* **2002**, *73*, 532−535.

(5) Quézel, P.; Barbero, M.; Loisel, R. *Artemisia molinieri*, espèce nouvelle de la flore française. *Bull. Soc. Bot. Fr*. **1966**, *113*, 523−531.

(6) Olivier, L.; Galland, J. P.; Maurin, H.; Roux, J. F. *Livre Rouge de la flore menacée de France. Tome 1. Espèces prioritaires*; Muséum National d'Histoire Naturelle, Conservatoire Botanique National de Porquerolles et Ministère de l'Environnement: Paris, France, 1995.

(7) Bohlmann, F.; Zdero, C. Notik uber die isolierung von zwei neunen sesquiterpènene aus *Artemisia molinieri*. *Chem. Ber*. **1975**, *108*, 2153−2155.

(8) Stangl, R.; Greger, H. Monoterpene und Systematik der Gattung *Artemisia* (*Asteraceae, Anthemidaceae*). *Plant Syst. Evol*. **1980**, *136*, 125−136.
(9) Kalemba, D.; Raska, A.; Kurowka, A.; Gora, J. Repellent and insecticidal activity of essential oils of *Artemisia asiatica* and *Artemisia molinieri*. *Pestycydy (Warsaw)* **1998**, *1*, 5−10.
(10) Carnat, A. P.; Lamaison, J. L.; Gueugnot, J. Composition of the essential oil of *Artemisia molinieri* Quézel, Barbero et R. Loisel. *J. Essent. Oil Res*. **1992**, *4*, 635−637.
(11) Swiader, K.; Lamer-Zarawska, E. Flavonoids of rare *Artemisia* species and their antifungal properties. *Fitoterapia* **1996**, *67*, 77−78.
(12) Budincevic, M.; Vrbaski, Z.; Turkulov, J.; Dimic, E. Antioxidant activity of *Oenothera biennis* L. *Fett. Wiss. Technol*. **1995**, *97*, 277−280.
(13) AFNOR (Association Française de Normalisation). *Huiles Essentielles*, 5th ed.; AFNOR: Paris, France, 1986.
(14) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatrography/Mass Spectroscopy*; Allured Publishing: Carol Stream, IL, 1995.
(15) Jennings, W.; Shibamoto, T. *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic Press: New York, 1980.
(16) Belaiche, P. *Traité de Phytothérapie et d'aromathérapie, Tome I: l'aromatogramme*; Maloine: Paris, France, 1979.
(17) Masotti, V.; Viano, J.; Dherbomez, M.; Letourneux, Y.; Gaydou, E. M. Phytochemical and antimicrobial studies on *Xylopia aethiopica*. *Fitoterapia* **1998**, *69*, 461−462.
(18) Mantle, D.; Anderton, J. G.; Falkous, G.; Barnes, M.; Jones, P.; Perry, E. K. Comparison of methods for determination of total antioxidant status: application and analysis of medicinal plant essential oils. *Comp. Biochem. Phys. B* **1998**, *121*, 385−391.
(19) Zang, P.; Omaye, S. T. Antioxidant and prooxidant roles for β-carotene, α-tocopherol and ascorbic acid in human lung cells. *Toxicol. in Vitro* **2001**, *15*, 13−24.
(20) Johnson, M. A.; Croteau, R. Biosynthesis of ascaridole: iodide peroxidase-catalyzed synthesis of a monoterpene endoperoxide in soluble extracts of *Chenopodium ambrosioides* fruit. *Arch. Biochem. Biophys*. **1984**, *235*, 254−266.
(21) Ruberto, G.; Baratta, M. T. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem*. **2000**, *69*, 167−174.
(22) Adegoke, G. O.; Iwahashi, H.; Komatsu, Y.; Obuchi, K.; Iwahashi, Y. Inhibition of food spoilage yeasts and aflatoxigenic moulds by monoterpenes of the spice *Aframomum danielli*. *Flavour Fragrance J.* **2000**, *15*, 147−150.
(23) Deans, S. G.; Svoboda, K. P.; Kennedy, A. I. Biological activity of plant volatile oils and their constituents. *Planta Med.* **1989**, *55*, 588.
(24) Pare, Paul W.; Zajicek, J.; Ferracini, V. L.; Melo, I. S. Antifungal terpenoids from *Chenopodium ambrosioides*. *Biochem. Syst. Ecol.* **1993**, *21*, 649−53.
(25) Delgadillo, G. M. T. Nontoxic insecticide, bactericide, and fungicide preparations containing ascaridol from *Chenopodium graveolens* or *Teloxys graveolens*. PCT Int. Appl. 2001; 5.
(26) Jimenez-Osornio, F. M. V. Z. J.; Kumamoto, J.; Wasser, C. Allelopathic activity of *Chenopodium ambrosioides* L. *Biochem. Syst. Ecol.* **1996**, *24*, 195−205.
(27) Belousova, N. I.; Dmitruk, S. E.; Khan, V. A. Essential oils in *Ledum* L.: antifungal properties. *Khim.-Farm. Zh.* **1989**, *23*, 317−319.

Received for review June 11, 2003. Revised manuscript received August 8, 2003. Accepted August 17, 2003.

JF034621Y