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An Ascaridole Containing Essential Oil of the *Achillea millefolium* L. Complex Growing Wild in Northern Greece

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ABSTRACT: The essential oil of the *Achillea millefolium* L. complex growing wild in Northern Greece was analyzed by GC and GC/MS. Several monoterpenes and sesquiterpenes were identified, although the main component was ascaridole (47.2%). Lesser amounts of 1,8-cineole (10.5%), p-cymene (7.4%), α-terpinene (7.0%) and camphor (8.1%) were also found.

KEY WORD INDEX: *Achillea millefolium* complex, Asteraceae, essential oil composition, ascaridole.

INTRODUCTION: *Achillea millefolium* has been used in the traditional medicine in Greece since the Trojan War, during which Achilles is said to have used it; hence its name. The herb has been used internally as a tea and externally in lotions and ointments. The essential oil is reported to lower the body temperature of rats (1), to increase the whitening properties of face creams and to possess disinfectant properties (2).

The *A. millefolium* L. complex is a group of difficult to separate species or subspecies of the family Asteraceae. Oswiecimska investigated several *Achillea* species growing in Eastern Europe and found that the *A. millefolium* was prochamazulene-free (3). Maffei et al. analyzed the oil of the five *Achillea* species growing in the Northwest Italian Alps and stated that the absence of proazulene compounds in the oil was related to the diploid chromosome number of the species investigated (4). Lawrence stated in a review that the chamazulene content of the oil of *A. millefolium* varies from 5.0% to 33.2% (5). Recently Bélanger et al. found that the oil of *A. millefolium* cultivated in Québec contained 34.6% chamazulene (6). Other main compounds in the essential oil are monoterpene hydrocarbons, oxygen-containing compounds (1,8-cineole, camphor, terpinen-4-ol and borneol) as

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well as sesquiterpenes (germacrene D, caryophyllene oxide) (5). We found it of interest to investigate the essential oil of the *A. millefolium* complex growing wild in Northern Greece.

EXPERIMENTAL: The plant material was collected on the Chalkidiki peninsula near Thessaloniki and air-dried. The botanical identification was performed by Dr. E. Drosos, Laboratory of Systematic Botany of the Aristotelian University and a voucher specimen has been deposited there. The dried plant material was subjected to hydrodistillation for three hours using a Clevenger-type apparatus, as described in the European Pharmacopoeia (7).

GC—The analyses were performed using a dual channel gas chromatograph CP 9000 (Chrompack Nederland BV, Middelburg, The Netherlands) equipped with FID and connected with two chromatographic processors, Chromatopac C-R3A (Shimadzu Corp., Kyoto, Japan). A DB-Wax fused-silica column, 60 m x 0.25 mm i.d., (film thickness 0.25 µm) and a DB-1 fused-silica column, 60 m x 0.25 mm i.d. (film thickness 0.25 µm) (J&W Scientific Inc., Rancho Cordova, CA, USA) were used. By means of a twin hole ferule (Chrompack Nederland) connected with the injector, the injected sample was split, and simultaneously one half was analyzed on one GC column, the other half on the other one. Gas chromatograph, oven temp programmed: 45-230°C at 3°C/min; injector temp: 220°C; detector temp: 240°C; carrier gas: nitrogen, pressure 150 kPa. The percentage composition was computed from the GC peaks without using correction factors.

GC/MS—GC/MS data were obtained on a gas chromatograph, Packard model 438 A, equipped with a fused-silica column 50 m x 0.22 mm i.d., coated with CP-Sil 5 cb (chemically bonded), film thickness 0.13 mm (Chrompack Nederland) and interfaced with a Finnigan MAT 700 ion trap detector (ITD); software version 3.0 (Finnigan MAT, San Jose, CA, USA). Conditions were as follows: GC oven temperature: as above; transfer line: 250°C; carrier gas: helium, pressure 150 kPa; split sampling technique: ratio 1:40; scan range 40-250 u; scan time, 1s.

The identity of the components was assigned by comparison of their mass spectra and retention times on the two columns of different polarities, mentioned under GC analysis, with those of components of reference oils and authentic compounds.

RESULTS AND DISCUSSION: The yield of oil was 1.3% after one hour of distillation, 1.5% after 2 h and 1.6% after 3 h. No increase in yield was observed for the following 2 h. The average oil composition is listed in Table I, in which the identified compounds are given, representing about 98% of the oil. The main compound was surprisingly ascaridole (47.2%) and an ascaridole isomer (2.1%). Other main components were 1,8-cineole (10.5%), camphor (8.1%), p-cymene (7.4%) and α-terpinene (7.0%). During GC of ascaridole, two peaks were always observed on the chromatogram, the second one was usually rather small. This small compound, which is also found in authentic ascaridole samples, represented an ascaridole isomer, according to the MS. When comparing the composition of the essential oil of the *Achillea millefolium* complex growing wild in Northern Greece with other oils of *A. millefolium*, the occurrence of relatively large amounts of ascaridole is remarkable.

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Table I. Percentage composition of identified compounds of the essential oil of *Achillea millefolium* L. growing wild in Northern Greece

Compound	Percentage	Compound	Percentage
artemisia triene	0.4	isoborneol	2.0
tricyclene	t	terpinen-4-ol	1.1
α-thujene	t	α-terpineol	0.5
α-pinene	0.4	cis-verbenone	0.2
camphene	0.9	ascaridole	47.2
octen-3-ol	t	thujyl isobutyrate	1.4
sabinene	0.4	geranial	0.3
β-pinene	0.3	chrysanthenyl acetate	t
myrcene	t	ascaridole isomer	2.1
α-phellandrene	t	carvacrol	t
δ-4-carene	t	thymol	0.1
α-terpinene	7.0	cis-jasmone	0.8
p-cymene	7.4	α-copaene	t
1,8-cineole	10.5	β-cubebene	0.1
limonene	0.1	β-elemene	t
artemisia ketone	t	β-caryophyllene	0.4
yomogi alcohol	1.7	(E)-β-farnesene	t
γ-terpinene	0.3	α-humulene	t
trans-sabinene hydrate	0.4	α-curcumene	t
artemisia alcohol	0.5	germacrene D	t
terpinolene	0.3	α-muurolene	t
linalool	0.5	γ-cadinene	t
α-thujone	1.5	δ-cadinene	t
β-thujone	0.2	(E)-nerolidol	t
cis-sabinene hydrate	0.5	β-caryophyllene oxide	0.2
camphor	8.1	T-cadinol	0.1
lavandulol	0.1	α-cadinol	0.1
cis-chrysanthenol	0.2	α-bisabolol	0.1

* Data given in order of increasing retention times on the DB-Wax column
t = trace (≤ 0.01%)

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