J. Essent. Oil Res., **9**, 541-544 (Sep/Oct 1997)

RESEARCH REPORT

Comparisons of the Leaf Oils of *Juniperus drupacea* Labill. from Greece, Turkey and the Crimea

Robert P. Adams
Plant Biotechnology Center, Baylor University, PO Box 669, Gruver, TX 79040 USA

Abstract

The composition of the leaf oils of *Juniperus drupacea* from Greece were analyzed and compared to literature reports on oils from the Crimea and Turkey. Both the Greek and the literature reports were in agreement that the major component is limonene (46-56%). Two other monoterpene hydrocarbons, α-pinene and δ-3-carene showed east-west geographic variation ranging from 5.1-22.1% and 7.0-22.3%, respectively. A total of 71 compounds were identified in the Greek oil.

Key Word Index

Juniperus drupacea, Cupressaceae, essential oil composition, limonene, α-pinene, δ-3-carene, geographical variation.

Introduction

Juniperus drupacea Labill. has a very limited distribution in the eastern Mediterrean region with two populations in Greece and other populations in Turkey, Syria and Lebanon (1). *J. drupacea* is very unusual and has been classified as a separate genus, *Arceuthos*, but recently, Adams and Demeke (2), using DNA fingerprinting confirmed its close relationship with *J. oxcedrus* L. and argue for its continued placement in the genus *Juniperus*, section *caryocedrus*.

A few reports have been made on the composition of the leaf oil. Horster (3) reported on the monoterpenes and Akimov et al. (4) reported on the leaf oil from the Crimea (see Table I). Sakar (5) reported on the leaf oil composition from plants from Turkey (see Table I). Recently, the antimicrobial acitivities of the leaf oil of *J. drupacea* were reported (6), but only α-terpineol was identified. The purpose of this report is to present a detailed analysis of the leaf oil of *J. drupacea* from Greece and compare this composition with the literature reports from Turkey and the Crimea.

Experimental

Foliage was collected (18 km. E. of Tripolis, Greece, R. P. Adams, 5651,5652) and voucher specimens are deposited at the BAYLU herbarium. The volatile leaf oils were isolated by steam distillation (200 g foliage, FW) using a circulatory Clevenger-type apparatus (7) for 2 h. The oil samples were concentrated (diethyl ether trap removed when collected in a Clevenger trap) with nitrogen and stored at –20°C until analyzed. Mass spectra were recorded with a Finnigan Ion Trap (ITD) mass spectrometer, model 800, directly coupled to a Varian 6500 gas chromatograph, using a 30 m x 0.25 mm J&W DB-5 (0.25 μm

Received: March 1996

Table I. Comparison (percentage) of leaf oils of *Juniperus drupacea* from Greece, Turkey (5) and the Crimea (4)

RI	Compound	Greece		Turkey (5)	Crimea (4)
		5651	5652		
854	(E)-2-hexenal	0.2	0.1	-	-
926	tricyclene	t	t	-	-
931	α-thujene	t	t	-	0.5
939	α-pinene	5.1	3.5	14.3	22.1
951	α-fenchene	1.2	0.6	0.5	-
953	camphene	-	-	-	0.6
973	terpene	0.8	0.3	-	-
976	sabinene	0.4	0.5	-	0.7
980	β-pinene	0.6	0.3	0.7	0.2
991	myrcene	3.0	2.7	3.5	6.7
1001	δ-2-carene	t	0.1	-	-
1005	α-phellandrene	t	0.1	-	-
1011	δ-3-carene	22.3	13.5	13.2	7.0
1018	α-terpinene	t	0.1	-	-
1022	o-cymene	t	0.3	-	-
1026	p-cymene	0.6	0.3	-	0.2
1031	limonene	46.7	48.4	55.6	50.3
1031	β-phellandrene	-	t	-	-
1033	1,8-cineole	-	-	4.3	-
1062	γ-terpinene	0.1	0.1	0.3	0.7
1068	cis-sabinene hydrate (cis rel. to OH vs. IPP)	t	0.1	-	-
1082	m-cymenene	t	t	-	-
1086	p-mentha-2,4(8)-diene	t	0.1	-	-
1088	terpinolene	0.8	0.8	0.8	1.1
1097	trans-sabinene hydrate (trans rel.to OH vs. IPP)-	t	t	-	-
1098	linalool	t	0.1	0.2	-
1099	perillene	t	t	-	-
1111	p-mentha-1,3,8-triene	t	t	-	-
1114	trans-thujone (=β-thujone)	-	t	-	-
1121	cis-p-menth-2-en-1-ol	-	0.1	-	-
1125	α-campholenal	t	t	-	-
1134	cis-limonene oxide	0.5	0.2	-	-
1139	trans-limonene oxide	0.4	0.2	-	-
1144	cis-verbenol	-	t	-	-
1159	p-mentha-1,5-dien-8-ol	0.8	0.4	-	-
1166	δ-terpineol	0.2	0.1	-	-
1177	terpinen-4-ol	0.6	0.5	0.2	-
1180	m-cymen-8-ol	0.5	0.3	-	-
1183	p-cymen-8-ol	0.2	0.2	-	-
1189	α-terpineol	0.5	1.1	-	-
1193	cis-dihydrocarvone	0.1	0.1	-	-
1204	verbenone	0.3	0.1	-	-
1217	trans-carveol	0.2	0.2	-	-
1229	cis-carveol	0.1	0.2	-	-
1242	carvone	0.3	0.2	-	-
1252	piperitone	t	0.2	-	-

Table I. Continued

RI	Compound	Greece 5651	Greece 5652	Turkey (5)	Crimea (4)
1351	α-cubebene	0.7	0.5	0.3	-
1376	α-copaene	0.6	0.7	0.1	-
1384	β-bourbonene	t	0.1	-	-
1390	β-cubebene	t	t	-	-
1418	β-caryophyllene	0.1	0.5	0.6	-
1429	cis-thujopsene	t	0.1	-	-
1454	α-humulene	0.1	0.6	0.3	-
1458	(E)-β-farnesene	t	t	-	-
1463	cis-muurolo-4(14),5-diene	0.1	0.1	-	-
1477	γ-muurolene	t	-	-	-
1480	germacrene D	0.4	0.1	-	-
1483	ar-curcumene	1.9	3.6	-	-
1495	β-alaskene*	0.4	0.3	-	-
1499	α-muurolene	t	0.2	-	-
1502	cuparene	t	0.1	-	-
1509	β-bisabolene	t	-	-	-
1513	α-alaskene	0.4	0.2	-	-
1513	γ-cadinene	0.2	1.6	0.2	-
1524	δ-cadinene	0.1	0.4	0.4	-
1552	sesquiterpene alcohol	0.5	0.9	-	-
1581	caryophyllene oxide	0.5	0.9	-	-
1596	cedrol	t	t	-	-
1606	humulene epoxide II	0.3	-	-	-
1610	sesquiterpene alcohol	0.6	1.0	-	-
1640	epi-α-cadinol (=T-cadinol)	0.2	0.8	-	-
1653	sesquiterpene alcohol	1.1	1.6	-	-
1684	sesquiterpene alcohol	0.6	1.1	-	-
1989	manoyl oxide	2.7	2.6	-	-
2010	manoyl oxide <13-epi->	t	0.1	-	-
2054	abietatriene	t	t	-	-

Compounds are listed in order of their elution from a DB5 column. RI = Retention indices (Kovat's Indices) on DB-5 (temperature programmed 60°-240°C at 3°C/min). * = tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported

coating thickness) fused silica capillary column (see reference 8 for operating details). Identifications were made by library searches of our volatile oil library, LIBR(TP) (8) using the Finnigan library search coupled with the standardized retention indices of reference compounds.

Results and Discussion

Oil yields ranged from 0.17-0.18% (2 h) and 0.38-0.44% (24 h) [oil wt./extracted, oven dried foliage weight]. The oils from all locations were dominated by limonene (Table I) which ranged from 46.7% to 55.6%. Other major components are α-pinene, myrcene, δ-3-carene, ar-curcumene, and manoyl oxide. There appears to be some geographic variation in α-pinene and myrcene (increasing from Greece to Crimea, i.e., west to east) and δ-3-carene (decreasing from Greece to Crimea). The presence of a considerable amount of manoyl oxide (2.6-2.7%) has not been previously reported. That is likely due to the perviously limited analyses at the high temperature needed to elute diterpenes. Horster (3) reported only monoterpene data. He apparently misidentified limonene as β-phellandrene, because he reported *J. drupacea* as having from 41.5% to 73.8% β-phellandrene. Because β-phellandrene is a trace

compound (Table I) and it elutes near limonene on many columns, it seems likely that he should have reported 41.5-73.8% limonene.

Horster (3), using monoterpene data, argued for the placing of *J. drupacea* in section *Oxycedrus* [= section *Juniperus*, see (2)]. However, the DNA data and female cone morphology clearly show *J. drupacea* so distinct from section *Juniperus* (cf. *J. oxycedrus*), that continued recoginition of *J. drupacea* in a mono-typic section (*caryocedrus*) is justified.

There were five unidentifed compounds (larger than 0.5% of the total oil) [ITMS, m/z (rel. int.): KI 973, 41(20), 51(13), 65(10), 77(13), 91(37), 105(8), 119(100), 134(12), terpene; KI 1552, 41(100), 55(36), 67(20), 79(50), 91(57), 105(50), 123(54), 131(34), 146(30), 159(25), 177(12), 187(10), 205(7), 220(10), sesquiterpene; KI 1610, 41(100), 55(38), 67(22), 79(48), 91(55), 105(53), 120(30), 131(50), 145(28), 159(42), 177(15), 187(15), 205(10), sesquiterpene; KI 1653, 41(100), 55(23), 67(32), 81(30), 91(47), 105(37), 117(42), 131(33), 145(15), 159(63), 177(23), 187(13), 202(19), 220(25), sesquiterpene; KI 1684, 41(100), 55(33), 67(29), 79(35), 91(45), 105(39), 117(20), 131(31), 145(15), 159(63), 177(10), 187(14), 202(21), 220(11), sesquiterpene; isomeric to KI 1653.

Acknowledgments

This research supported by funds from Baylor Univeristy.

References

1. K. Browicz, *Chorology of trees and shrubs in south-west Asia and adjacent regions*. Polish Sci. Publ., Warsaw, Poland (1982).
2. R. P. Adams, *Systematic relationships of Juniperus based on random amplified polymorphic DNAs (RAPDs)*. Taxon, **42,** 53-571 (1993).
3. V. H. Horster, *Comparison of the monoterpene fractions of J. drupacea and J. oxycedrus*. Planta Med., **26**, 113-118 (1974).
4. Y. A. Akimov, S. I. Kuznetsov, G. I. Nilov, N. N. Chirkina, A. P. Krylova and R. M. Litvinenko, *Essential oils of Junipers from ancient mediterranean region. Composition, properties and prospectives of use*. Tr. Nikitsk. Botan. Sad., **69**, 79-93 (1976).
5. M. K. Sakar, *Composition of the oil of Juniperus drupacea leaves*. Fitoterapia, **56**, 360-361 (1985).
6. V. Stassi, E. Verykokidou, A. Loukis, C. Harvala and S. Philianos, *The antimicrobial acitivity of the essential oils of four Juniperus species growing wild in Greece*. Flav. Fragr. J., **11**, 71-74 (1996).
7. R. P. Adams, *Cedar wood oil - Analysis and properties*. In: *Modern Methods of Plant Analysis: Oils and Waxes*. Edits., H. F. Linskins and J. F. Jackson, Springler-Verlag, Berlin (1991).
8. R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/ Ion Trap Mass Spectroscopy*. Allured Publ. Corp., Carol Stream, IL (1995).