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RESEARCH REPORT

The Leaf Oil of *Juniperus gracilior* Pilger var. *urbaniana* (Pilger & Ekman) R.P.Adams: Comparison with Other Caribbean *Juniperus* Species

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Abstract

The composition of the leaf oil of *Juniperus gracilior* var. *urbaniana* from Haiti was analyzed and compared to the composition of the other Caribbean junipers. The oil was dominated by bornyl acetate (38.4%) with moderate amounts of limonene (12.2%), sabinene (12.2%) and camphor (5.8%). *J. gracilior* var. *urbaniana* appeared to be most closely related to *J. gracilior* var. *ekmanii* and *J. gracilior*. The evolution of the Caribbean junipers is discussed.

Key Word Index

Juniperus gracilior var. *urbaniana*, Cupressaceae, essential oil composition, Caribbean junipers, evolution, bornyl acetate, limonene, sabinene, camphor.

Introduction

The genus *Juniperus* is divided into three sections: *Caryocedrus, Juniperus* and *Sabina* (1). The Caribbean junipers have been the focus of numerous studies, beginning with Linnaeus (2) who described only three junipers from the New World (*J. virginiana*, "Virginia and Carolina;" *J. barbadensis*, "America;" and *J. bermudiana*, "America"). However, Hemsley (3) equated *J. barbadensis* with *J. bermudiana*, adopting *J. bermudiana* as the name for all of the Caribbean junipers. Sargent (4) recognized *J. barbadensis* and said it occurred along the Atlantic coast of Georgia and Florida as well as "on the Bahamas, San Domingo (Dominican Republic), mountains of Jamaica and on Antigua." Britton (5) recognized *J. lucayana* in the Bahamas and reserved *J. barbadensis* for the plants of southern Georgia, Florida, and the rest of the Caribbean. Pilger (6) equated *J. bermudiana* and *J. barbadensis*, but used *J. barbadensis* for the name of the common juniper of the Caribbean on the grounds that it was listed first by Linnaeus (2). Florin (7) reviewed the junipers of the Caribbean and recognized 5 species: *J. saxicola* from Cuba; *J. lucayana* from Cuba, Haiti, Jamaica and the Bahamas; *J. gracilior* from Haiti and Dominican Republic; *J. ekmanii* from Haiti; and *J. urbaniana* from Haiti. Carabia (8) recognized *J. barbadensis* throughout the Caribbean, *J. bermudiana* on Bermuda, and *J. virginiana* in the United States. Gillis (9) treated the Bahamian junipers as *J. bermudiana*. Correll & Correll (10) recognized the juniper of the Bahamas as *J. barbadensis*.

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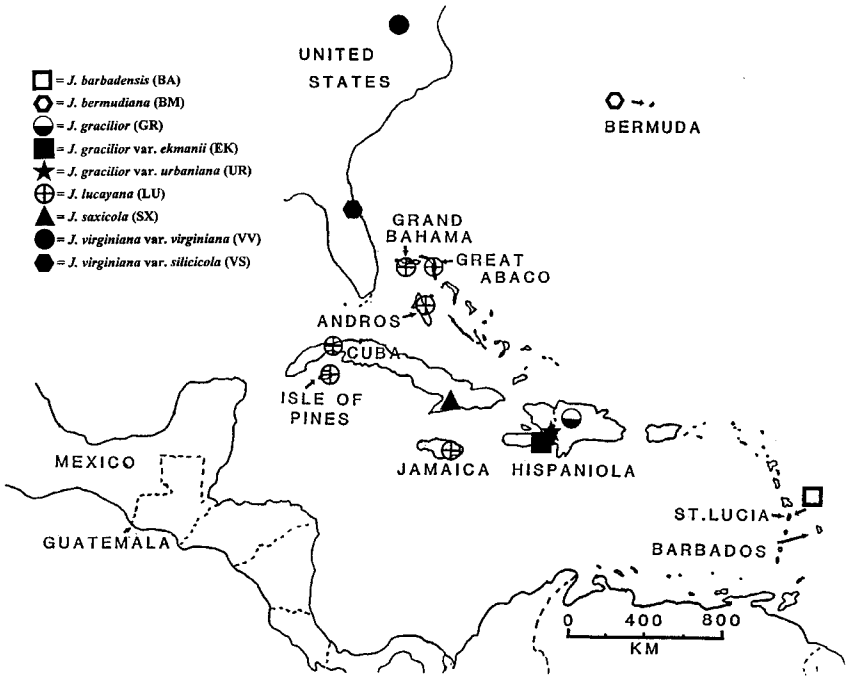

Figure 1. Study area of the Caribbean with population locations. Additional populations of *Juniperus barbadensis* var. *lucayana* (not sampled) occur on a few of the islands of the Bahamas and in eastern Cuba

Morphologically all the Caribbean species of *Juniperus*, except *J. bermudiana* and *J. saxicola*, are very similar and difficult to distinguish. All the Caribbean junipers are in section Sabina and are also in the entire leaf-margin-series. Based on DNA fingerprinting, the entire leaf margin and denticulate-series appear to represent monophyletic groups (1). The variable nature of leaves, even on a single branch, has resulted in confusion in the taxonomy of the Caribbean junipers. It is unlikely that the systematic relationships could ever be determined based solely on morphology. However, each taxon possesses a quite different pattern of leaf oil composition and the taxa are readily separated using such data (11-14). Examination of both the leaf oils and morphology of natural populations of *J. virginiana* and *J. silicicola* (15) indicated that these taxa are conspecific and the juniper of the coastal foredunes of the southeastern United States (*J. silicicola*) was therefore maintained as a variety of *J. virginiana* (i.e., *J. virginiana* var. *silicicola*). In previous studies of the Caribbean junipers, the oil compositions of *J. barbadensis, J. bermudiana, J. ekmanii, J. gracilior, J. lucayana, J. saxicola, J. virginiana* and *J. virginiana* var. *silicicola* (Small) E. Murray have been reported and the systematic relationships examined among the taxa (11-14,16).

Recently, Adams (1) re-examined the morphology and oils of all the Caribbean junipers (except *J. gracilior* var. *urbaniana*) and recognized the following taxa: *J. barbadensis* L. (St. Lucia, BWI), *J. barbadensis* var. *lucayana* (Britton) R.P.Adams (Bahamas, Cuba, Jamaica, Hispaniola), J. gracilior Pilger (Hispaniola), *J. gracilior* var. ekmanii (Florin) R.P.Adams (Hispaniola), *J. gracilior* var. *urbaniana* (Pilger & Ekman) R.P.Adams (Hispaniola) and *J. saxicola* Britton & Wilson (Cuba). In addition, *J. bermudiana* was retained as the name for the juniper endemic to Bermuda.

After several unsuccessful attempts to collect materials from *J. gracilior* var. *urbaniana*, the taxon was recently discovered within a few meters of the site that Walter Judd collected this prostrate shrub

in 1984 (personal communication). In this paper, the oil composition of the leaves from this taxon is reported and compared with the oils of the other Caribbean junipers.

Experimental

Figure 1 shows the populations sampled. Taxon, acronym, collector number, location: *J. barbadensis* (BA), Adams 5367-5371; Petit Piton, St. Lucia, BWI; *J. bermudiana* (BM), Adams 2553-2567, Bermuda; Haiti; *J. barbadensis* var. *lucayana*: (LG) Adams 2686-2695, 55 km s of Marsh Harbour, Great Abaco, Bahamas; (LA) Adams 2696-2705, 5 km s of Andros Town, Andros Island, Bahamas; (LB) Adams 2706-2715, 3 km north of Freeport, Grand Bahama Island, Bahamas; (LC) Adams 5259-5280, Havana Botanical Garden (seed from Sierra de Nipe), Cuba; (LP) Adams 5281-5282, Havana Botanical Garden (seed from Isle de Pinos), Cuba; (LJ) Adams 2875-2884), Hardware Gap, Blue Mtns., St. Andrew Parish, Jamaica; *J. gracilior* (GR), Adams 2785-2794, w of Constanza, Dominican Republic; *J. gracilior* var. *ekmanii* (EK), Adams 3106-3107, Pic la Selle, *J. gracilior* var. *urbaniana* (UR) Adams 7656-7658, Pic la Selle, Haiti; *J. saxicola* (SX) Adams 5284-5285, w slope of Pico Turquino, Prov. Granma/ Santiago de Cuba boundary, Cuba; *J. virginiana* (VV) Adams 2409-2423; 16 km e of Dulles Airport on highway I495, Fairfax Co., Virginia; *J. virginiana* var. *silicicola* (VS) Adams 2775-2784, 1.6 km e of the jct. of US highway 1 and E. Halifax St., Oak Hill, Volusia Co., Florida). Herbarium vouchers for all of the aforementioned collections are deposited at SRCG.

The volatile leaf oils were isolated by steam distillation (200 g foliage, FW) using a circulatory Clevenger-type apparatus (18) for 2 h. The oil samples were concentrated (diethyl ether trap removed when collected in the lighter than water oil 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 J&W DB5, 0.26 mm x 30 m, 0.25 μm coating thickness, fused silica capillary column (see reference 19 for operating details). Identifications were made by library searches of our volatile oil library, LIBR(TP) (19) using the Finnigan library search coupled with the standardized retention indices of reference compounds.

Results and Discussion

Oil yields from *J. gracilior* var. *urbaniana* ranged from 0.7-1.45% (2 h) [oil wt/oven dried foliage weight]. The oil was dominated by bornyl acetate with moderate amounts of limonene, terpinen-4-ol, camphor, borneol, and sabinene (Table I). The oil was very similar to the leaf oils of *J. gracilior* (GR) and *J. gracilior* var. *ekmanii* (EK).

There was only one new unidentified compound (>0.5% of the total oil) [ITMS, m/z (rel. int.)]: KI 1609, 43(100), 55(5), 67(7), 81(7), 93(18), 109(17), 119(21), 134(10), 152(5), 195(6), 196(1), terpene acetate. The other unidentified compounds listed in Table I have been previously discussed (11-14,16).

In order to assess the relationships among the junipers of the West Indies, weighted Gower metric similarities (1) were computed among the 14 OTUs using F-1 weights (F ratio from ANOVA) for 79 terpenoids. The resulting similarity matrix was then factored using principal coordinate analysis (PCO). The first 7 eigenroots (coordinates) accounted for 23.5, 17.9, 12.1, 9.6, 7.3, 6.2, and 5.5% (total of 82.2%) of the variation among the 14 OTUs. The first coordinate (24%) separated the junipers of Hispaniola (EK, GR, UR) from all the other taxa in the study (Figure 2). The second coordinate (18%) separated the two varieties of *J. virginiana* (VV, VS) from all the other taxa (Figure 2). The third coordinate (12%) separated *J. barbadensis* (BA) and *J. saxicola* (SX) from the other taxa. Coordinate 4 (not shown) separated *J. bermudiana* from *J. saxicola* and coordinate 5 separated various populations of *J. barbadensis* var. *lucayana*. The two varieties of *J. virginiana* were separated on coordinate 6 (not shown). The minimum spanning network that is superimposed (Figure 2) is based on the similarity measures and, thus, is useful in sensing the distortion of viewing these 14 OTUs in a 3-dimensional ordination.

Table I. Percentage composition of the leaf oil *Juniperus gracilior* var. *urbaniana*, compared with the other Caribbean junipers

RI	Compound	BA	LJ	LB	SX	BM	UR	EK	GR	VS	VV
	Oil Yield(%)	0.6	0.6	0.2	0.3	0.3	1.1	2.2	0.8	0.4	0.2
901	unknown	-	-	t	-	-	-	-	0.8	-	-
912	unknown	-	-	t	-	-	-	-	0.8	-	t
926	tricyclene	t	t	t	t	t	1.5	1.2	t	t	t
931	α-thujene	0.9	0.6	0.5	1.1	t	0.8	0.7	1.0	t	t
939	α-pinene	7.4	49.1	33.0	24.4	22.3	1.6	1.3	1.8	2.4	1.4
953	camphene	t	t	t	t	0.7	0.6	1.9	1.2	t	t
976	sabinene	31.0	9.7	8.3	25.2	2.8	12.2	5.0	10.1	t	6.7
978	1-octen-3-ol		t	t	-	1.0	-	t	t	0.9	-
980	β-pinene	t	1.1	1.2	t	0.6	t	t	t	t	t
991	myrcene	3.8	3.2	4.0	2.7	2.9	3.6	2.5	1.9	0.9	0.9
1001	δ-2-carene		t	t	-	-	0.6	t	-	t	t
1005	α-phellandrene	t	-	-	t	t	t	-	t	-	-
1011	δ-3-carene		-	-	t	t	t	-	-	t	t
1018	α-terpinene	1.7	t	t	2.4	t	1.4	0.9	1.7	t	t
1026	p-cymene	t	t	t	0.8	0.5	2.2	0.5	1.4	t	-
1031	limonene	34.2	25.9	18.0	2.6	35.3	12.2	9.6	7.3	33.3	8.9
1031	β-phellandrene	t	-	-	t	-	t	-	-	-	t
1050	(E)-β-ocimene	0.7	t	-	t	t	-	-	-	-	t
1062	γ-terpinene	2.7	0.8	0.7	3.7	0.7	2.8	1.7	3.5	t	t
1068	cis-sabinene hydrate (cis rel. to OH vs. IPP)	0.9	-	t	0.7	-	1.0	0.9	1.1	-	t
1088	terpinolene	1.2	1.0	0.8	1.2	0.8	0.9	0.6	0.9	t	0.5
1097	trans-sabinene hydrate	0.7	t	t	t	t	t	-	t	-	t
1098	linalool	-	-	t	-	1.1	-	0.6	2.6	1.5	4.4
1102	cis-thujone (=α-thujone)	-	-	-	-	t	-	1.6	2.0	-	-
1121	cis-pinene hydrate	t	-	-	-	-	0.5	0.5	0.8	-	-
1139	trans-pinocarveol	-	-	t	-	1.1	-	-	-	-	-
1140	trans-pinene hydrate	t	t	t	-	-	t	t	0.7	-	-
1143	camphor	-	t	t	2.8	6.5	1.9	5.8	1.1	t	3.7
1148	camphene hydrate	-	-	t	-	1.4	0.6	2.2	1.4	t	t
1165	borneol	t	t	t	-	2.1	2.0	5.1	2.0	-	0.8
1177	terpinen-4-ol	6.5	1.6	2.5	5.9	1.4	6.0	6.3	11.6	t	1.5
1183	p-cymen-8-ol	-	t	t	t	t	t	-	-	t	t
1189	α-terpineol	t	t	t	t	t	1.2	0.8	0.9	-	t
1193	myrtenal	-	-	t	-	0.7	-	-	-	t	t
1193	cis-piperitol	t	-	-	t	-	-	t	t	-	-
1195	methyl chavicol (=estragole)	-	-	-	-	-	-	-	-	0.5	t
1205	trans-piperitol	t	-	-	-	-	-	-	-	-	-
1228	citronellol	t	t	0.9	t	t	-	0.6	0.6	t	2.3
1242	carvone	t	t	t	-	1.0	-	t	t	t	t
1252	piperitone	-	-	0.6	-	-	1.1	-	t	t	t
1268	unknown	-	-	-	-	-	-	-	1.2	-	-

Table I. Continued

RI	Compound	BA	LJ	LB	SX	BM	UR	EK	GR	VS	VV
1285	bornyl acetate	t	0.6	4.1	t	3.7	38.4	43.9	35.7	t	2.1
1285	safrole	-	t	-	-	0.5	-	-	-	13.7	6.7
1297	trans-pinocarvyl acetate	t	-	t	-	0.8	-	-	t	-	-
1401	methyl eugenol	-	t	-	-	t	0.9	-	t	8.2	2.9
1418	(E)-caryophyllene (=β-)	t	t	t	t	t	t	t	t	t	t
1429	cis-thujopsene	t	-	t	t	2.1	-	t	t	-	-
1473	β-cadinene	-	-	t	-	-	t	t	t	t	t
1480	germacrene D	t	0.6	t	2.5	t	0.6	-	-	t	t
1493	epi-cubebol	-	-	0.9	-	-	-	-	-	-	-
1499	α-muurolene	t	t	-	t	t	t	t	t	t	t
1502	cuparene	t	-	-	-	-	-	-	-	-	-
1513	γ-cadinene	t	-	0.8	t	-	0.6	t	-	t	t
1514	cubebol	-	t	2.0	t	-	-	t	t	t	-
1524	δ-cadinene	t	t	0.7	t	t	0.6	t	t	0.7	0.8
1542	sesquiterpene alcohol	-	-	0.6	-	-	t	-	-	t	t
1549	elemol	t	t	t	1.8	t	0.7	-	-	2.1	8.2
1554	elemicin	-	-	-	-	-	t	t	t	-	t
1574	germacrene D-4-ol	t	-	1.9	-	-	-	-	-	0.9	t
1587	cadinol isomer 2	-	t	0.9	-	-	-	-	-	0.8	0.7
1596	widdrol	t	-	-	-	-	-	-	-	-	-
1596	cedrol	t	-	-	-	-	t	-	-	-	-
1606	β-oplopenone	t	t	2.2	t	t	t	-	-	0.7	2.0
1609	terpene acetate		-	-	-	-	0.7	-	-	-	-
1627	1-epi-cubenol	-	-	1.2	-	t	0.6	t	t	0.7	0.9
1630	γ-eudesmol	-	-	-	0.8	-	t	-	-	3.4	2.8
1640	epi-α-cadinol (=τ-cadinol)	-	t	-	-	-	-	t	t	2.0	t
1641	epi-α-muurolol (=τ-muurolol)	-	t	1.6	-	t	-	t	-	-	2.4
1649	β-eudesmol	-	-	-	1.2	-	t	-	-	2.8	1.7
1652	α-cadinol	t	-	-	t	-	-	-	-	-	-
1652	α-eudesmol	-	-	-	1.6	-	t	-	-	3.4	3.1
1684	sesquiterpene alcohol	t	0.5	2.3	1.3	t	t	t	t	-	-
1688	sesquiterpene alcohol	t	t	0.9	1.6	t	-	t	t	t	t
1733	oplopanone	t	t	t	-	-	-	t	-	-	-
1789	8-α-acetoxyelemol	-	-	-	t	-	-	-	-	1.8	3.5
2054	abietatriene	t	-	t	t	t	t	t	t	t	-
2056	manool	-	-	t	0.9	-	-	-	-	t	t
2080	abietadiene	t	-	t	t	0.7	-	-	t	-	-
2278	cis-totarol	0.6	t	t	t	-	t	-	-	-	-
2288	4-epi-abietal	t	-	-	8.9	-	-	-	-	t	t
2303	trans-totarol	t	t	t	1.9	2.0	t	-	5.8	t	t

BA = *J. barbadensis*, St. Lucia, BWI; LJ = *J. barbadensis* var. *lucayana*, Jamaica; LB = *J. barbadensis lucayana*, Bahama Islands; SX = *J. saxicola*, Cuba; BM = *J. bermudiana*, Bermuda; UR = *J. gracilior* var. *urbaniana*, Haiti; EK = *J. gracilior* var. *ekmanii*, Haiti; GR = *J. gracilior*, Dominican Republic; VS = *J. virginiana* var. *silicicola*, Florida, USA; and VV = *J. virginiana* var. *virginiana*, Washington, DC, USA. Volatile oil data for BA, LJ, LB, SX, BM, EK, GR, VS and VV were from our previous reports (11-14,16).
RI = Kovat's index on DB-5 (=SE54) column; t = trace (<0.5%); unidentified components <0.5% are not reported

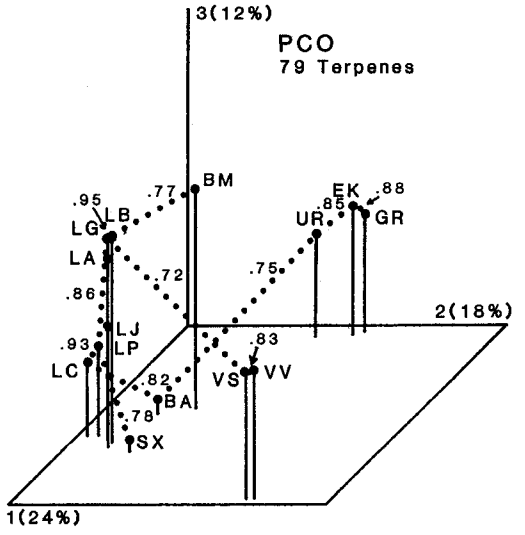

Figure 2. Principal coordinate analysis based on 79 terpenoids from the leaf oils. The individual components were weighted by F-1 (F from ANOVA of the 14 OTUs) in the similarity measure. The minimum spanning network (dashed line) distances are the similarities. The OTU codes are: BA = *Juniperus barbadensis*, St. Lucia; BM = *J. bermudiana*, Bermuda; EK = *J. gracilior* var. *ekmanii*, Haiti; GR = *J. gracilior*, Dominican Republic; LA, LB, LC, LG, LJ, and LP = *J. barbadensis* var. *lucayana*, from, respectively, Andros Island, Grand Bahama Island, Cuba (mainland), Jamaica, and Isle of Pines; SX = *J. saxicola*, Cuba; UR = *J. gracilior* var. *urbaniana*, Haiti; VV = *J. virginiana*, Washington, D.C.; and VS = *J. virginiana* var. *silicicola*, Florida, USA. The percentage number on each axis is the percent of the total variation among the OTUs accounted for by that axis. The numbers on the dotted lines are the similarities between OTUs

Several patterns are apparent from the chemical data. The island populations of *J. barbadensis* var. *lucayana* are very similar to each other (similarities range from 0.95 to 0.86, Figure 2). The three varieties of *J. gracilior* from Hispaniola form a distinct group that is characterized by the high concentration of bornyl acetate in these taxa. The *J. virginiana* varieties are also very similar to each other but quite distinct from other Caribbean taxa. *J. bermudiana* is distinct but most similar to the *J. barbadensis* var. *lucayana* populations. *J. barbadensis* is similar to *J. barbadensis* var. *lucayana* (0.82), but somewhat distinct. And finally, *J. saxicola*, although morphologically quite distinct in having only decurrent leaves, is clearly similar to the *J. barbadensis* complex.

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