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Chemosystematic Studies of the Caribbean Junipers Based on their Volatile Oils

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Key Word Index – *Juniperus bermudiana*; *J. ekmanii*; *J. gracilior*; *J. lucayana*; *J. silicicola*; *J. virginiana*; Cupressaceae; terpenoids; numerical analysis.

Abstract – Six taxa of smooth leaf margined junipers were collected from the West Indies, Bermuda and the south-eastern United States for chemosystematic analyses of their volatile oils. These taxa were *Juniperus bermudiana*, *J. ekmanii*, *J. gracilior*, *J. lucayana*, *J. silicicola* and *J. virginiana*. Three major groups were found: Hispaniola (*J. ekmanii*, *J. gracilior*); Bermuda/Bahamas (*J. bermudiana*, *J. lucayana*) and south-eastern United States (*J. silicicola*, *J. virginiana*). The Hispaniola junipers were found to be unique among the smooth leaf margined junipers of the Western Hemisphere in having a large percentage of bornyl acetate in the leaf oil composition. Systematic implications and possible routes of migration and evolution are discussed.

Introduction

The junipers of the Caribbean have been the subject of considerable nomenclatural chaos ever since the first species was described by Linnaeus. Zanoni [1] examined herbarium specimens of the Caribbean Island junipers and concluded that the "nomenclatural matter has not been resolved, and cannot be resolved by examination of herbarium specimens only".

There is little agreement as to how many juniper taxa are in the Caribbean. We have sampled junipers from most of the islands that have junipers with the exception of Cuba. Therefore, *Juniperus saxicola* Britt. & Wilson, endemic to Cuba, is excluded as well as *J. urbaniana* Pilger & Ekman, which is endemic to Haiti but has not yet been collected [2]. In general, the nomenclature of Florin [3] has been followed in this study. He recognized *J. saxicola* as endemic to Cuba, *J. lucayana* Britt. from Cuba, Haiti, Jamaica and the Bahama Islands, *J. gracilior* Pilger from Haiti and Dominican Republic, and *J. ekmanii* Florin and *J. urbaniana* as endemic to Haiti. *Juniperus bermudiana* L. is accepted as the correct name of the juniper from Bermuda [4].

Zanoni [1] cited Small [5] in the recognition of *J. silicicola* (Small) Bailey as the coastal juniper of the south-eastern United States and maintained the taxon separate from *J. virginiana* L. and distinct from the Caribbean Island junipers.

As a first step in our analyses we will present the volatile oil composition of the foliage of juniper species from the Caribbean and attempt to resolve some of the relationships among this group.

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Results

Oil yields from the Caribbean Island junipers were much lower than yields from junipers from the south-western United States [6]. Yields ranged from 1.4% dry foliage weight in *J. ekmanii* (EK, Table 1) to 0.2% in *J. lucayana*. Few compounds are unique within this group except *trans*-pinocarveol in *J. bermudiana* and an α-cadinol isomer in *J. virginiana*. The Caribbean junipers are obviously closely related in both oils and morphology. There are, however, some major shifts in composition. The two junipers from Hispaniola (*J. ekmanii* and *J. gracilior*) have a very high concentration of bornyl acetate. This pattern is unique among the smooth leaf margined junipers in the Western Hemisphere [7], which include *J. blancoi*, *J. horizontalis*, *J. silicicola*, *J. scopulorum* and *J. virginiana* in addition to all the Caribbean junipers. The Hispaniolan junipers also show oil similarities in per cent yield and levels of tricyclene, sabinene, limonene, dehydrocarveol (isomer 1), 4-terpineol

TABLE 1. COMPOSITION OF THE VOLATILE LEAF OILS OF CARIBBEAN JUNIPER SPECIES

	% Total oil*						
Yield/component	EK	GR	BM	LU	SI	VG	F ratio
% Yield	1.4	0.8	0.3	0.2	0.4	0.2	39.4
Unknown 1, *RRT* = 0.143	(T)	0.8	-	T	-	(T)	10.3
Unknown 2, *RRT* = 0.151	(T)	0.8	-	T	-	T	11.4
Tricyclene	1.9	1.4	T	T	T	T	196.2
α-Pinene	1.3	1.8	22.3	38.3	2.4	1.4	83.3
Camphene	1.9	1.2	0.7	0.6	T	T	38.9
Sabinene	5.0	10.1	2.8	1.1	T	6.7	9.9
β-Pinene	T	T	0.6	1.1	T	(T)	50.4
7-Octen-4-ol	T	T	1.0	T	0.9	-	28.8
Myrcene	2.5	1.9	2.9	4.3	0.9	0.9	170.8
α-Terpinene	0.9	1.7	T	T	T	T	14.8
p-Cymene	0.5	1.4	0.5	T	T	-	7.3
Limonene	9.6	7.3	35.3	26.9	33.3	18.9	21.0
γ-Terpinene	1.7	3.5	0.7	T	T	T	15.7
β-Terpineol isomer	0.9	1.1	-	T	-	-	69.6
Terpinolene	0.6	0.9	0.8	1.0	(T)	0.5	8.6
Linalool	(0.6)	2.6	1.1	1.8	1.5	4.4	7.9
Unknown 3, *RRT* = 0.337	1.6	2.0	(T)	-	-	-	57.4
Dehydrocarveol, isomer 1	0.5	0.8	-	-	-	-	23.4
Camphor	5.8	1.1	6.5	T	T	3.7	11.9
Trans-pinocarveol	-	-	1.1	-	-	-	74.7
Dehydrocarveol, isomer 2	(T)	0.7	-	(T)	-	-	14.7
Camphene hydrate	2.2	1.4	1.4	(T)	T	T	25.3
Borneol	5.1	2.0	2.1	T	-	0.8	15.8
4-Terpineol	6.2	11.6	1.4	1.0	T	1.5	20.1
Myrtenal	-	-	0.7	(T)	T	(T)	55.9
α-Terpineol (+ estragole)	0.8	0.9	T	T	0.5	T	13.0
Unknown 4, *RRT* = 0.426	(T)	1.2	-	-	-	-	12.1
Carvone	(T)	T	1.0	(T)	T	T	75.9
Citronellol	(0.6)	0.6	T	1.2	T	2.3	5.4
Unknown 5, *RRT* = 0.462	(T)	0.5	(T)	T	-	-	45.3
Isosafrole	-	-	(T)	(T)	3.6	6.7	72.6
Bornyl acetate	43.9	35.7	4.2	3.8	T	2.1	32.3
Safrole	-	(T)	-	(T)	13.7	10.9	13.6
Sabinyl acetate	(T)	(T)	0.7	T	-	-	2.0
Methyl eugenol	-	T	(T)	(T)	8.2	2.9	7.1
Thujopsene	(T)	T	2.1	(T)	(T)	T	33.9
Germacrene D	-	-	T	0.6	(T)	T	31.7
δ-Cadinene	T	T	T	0.6	0.7	0.8	3.1
Unknown 6, *RRT* = 0.692	-	-	-	0.6	(T)	(T)	15.8
Elemol (+ elemicin)	-	-	T	T	12.1	8.2	49.7
α-Cadinol, isomer	-	-	-	-	-	2.5	43.1
Unknown 7, *RRT* = 0.686	-	-	-	0.9	0.9	-	11.6
α-Cadinol, isomer	(T)	(T)	-	T	(0.8)	(0.7)	11.5
4-(2,6,6-Trimethyl-1-cyclohexane-l-yl-(*E*)-3-butene-2-one)	-	-	T	0.7	0.7	2.0	13.3
(Cubenol)	T	T	T	T	(0.7)	(0.9)	10.0
γ-Eudesmol	-	-	-	-	3.4	2.8	31.2
α-Cadinol, isomer 1	T	-	T	0.8	-	2.4	47.4
α-Cadinol, isomer 2	(T)	(T)	-	-	2.0	-	54.9
β-Eudesmol	-	-	-	-	2.8	1.7	31.1
α-Eudesmol	-	-	-	-	3.4	3.1	47.3
Unknown 8, *RRT* = 0.769	(T)	T	T	1.3	-	-	30.3
Unknown 9, *RRT* = 0.791	T	T	T	1.1	T	(T)	20.0
Acetate II, *RRT* = 0.860	-	-	-	-	1.8	3.5	42.9
Unknown 10, *RRT* = 1.037	-	(T)	0.7	T	-	-	9.3
Total No. of compounds	38	40	38	44	39	40	
No. of unique compounds	0	0	1	0	0	1	

*EK = *J. ekmanii*, GR = *J. gracilior*, BM = *J. bermudiana*, LU = *J. lucayana*, SI = *J. silicicola*, VG = *J. virginiana*. Compositional values in parentheses indicate that a component elutes at that retention time but no spectrum was obtained. Trace, T, indicates that the component was less than 0.5% of the total oil. Components are listed in order of their elution on SP2100. F ratio from ANOVA of the six taxa.

and bornyl acetate. *Juniperus bermudiana* and *J. lucayana* share similar concentrations of α-pinene and limonene but differ in camphor and thujopsene levels along with many smaller components. The oils of *J. silicicola* and *J. virginiana* are quite similar being characterized by large amounts of limonene, safrole, methyl eugenol and elemol. In addition, the aromatic methyl ethers derived from the phenylpropanoid pathway (isosafrole, safrole, methyl eugenol, and elemicin) are chiefly found in these two taxa.

Mass spectra of the structurally unknowns (greater than traces) are: compound **1**, *RRT*=0.143 (*m/z* (%)) MW 140?(2), 55 (100), 43 (80), 125(42), 6(40), 71(28), 93(26); compound **2**, *RRT*=0.151, MW 140(1), 55(100), 43(67), 125(43), 67(33), 71(27), 93(27), isomeric to compound **1**; compound **3**, *RRT*=0.337, MW 152(3), 43(100), 71(69), 81(46), 67(42), 79(31), 53(29), unsaturated terpene alcohol or ketone; compound **4**, *RRT*=0.426, MW 154(4), 41(100), 93(56), 84(42), 43(40), 81(29), terpene alcohol; compound **5**, *RRT*=0.462, MW 152(11), 43(100), 119(50), 91(31), 79(27), 105(24), unsaturated terpene alcohol or ketone; compound **6**, *RRT*=0.692, MW 223(3), 43(100), 95(58), 41(48), 55(46), 109(33), 93(33), sesquiterpene alcohol; compound **7**, *RRT*=0.686, MW 222(1), 43(100), 81(97), 41(76), 79(51), 93(46), sesquiterpene alcohol; compound **8**, *RRT*=0.769, MW 220(10), 43(100), 41(85), 91(43), 95(40), 55(46), 81(44), sesquiterpene alcohol; compound **9**, *RRT*=0.791, MW 220(14), 41(100), 43(65), 91(45), 55(38), 159(36), sesquiterpene alcohol, isomeric to compound **8**; compound **10**, *RRT*=1.037, MW 272(40), 41(100), 105(72), 43(68), 55(58), 136(54), 91(54), diterpene. For "Acetate II" see von Rudloff [8, 9] for detailed discussion.

Fig. 1 shows the six taxa plotted onto the first three principal coordinates. These three coordinates account for 37.3, 28.5 and 14.9% of the variation among taxa. The taxa are broken into four groups: Hispaniola junipers (*J. ekmanii* (EK), *J. gracilior* (GR)); south-eastern United States junipers (*J. silicicola* (SI), *K. virginiana* (VG)); Bahama juniper (*J. lucayana* (LU)) and the Bermuda juniper (*J. bermudiana* (BM)). Although it is not readily apparent from Fig. 1, *J. lucayana* (LU) and *J. bermudiana* (BM) are mutually most similar in their oils. The dashed line indicates the minimum spanning tree and shows a trend of differentiation from the United States mainland to the islands and then to Hispaniola.

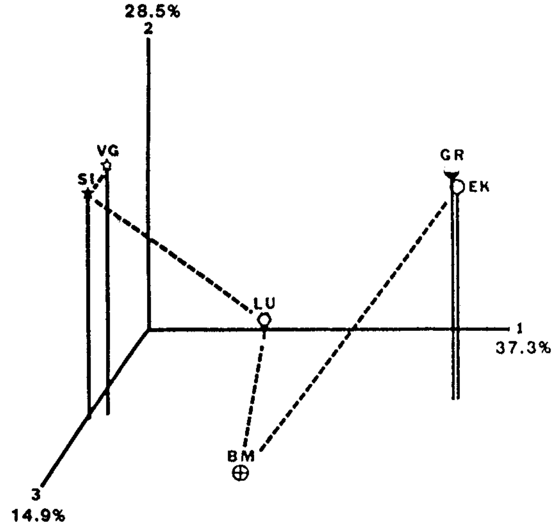

FIG. 1. PRINCIPAL COORDINATE ANALYSIS WITH A MINIMUM SPANNING TREE SUPERIMPOSED (DASHED LINE). Four groups are apparent: Hispaniola (*J. ekmanii, J. gracilior*); Bahama Island (*J. lucayana*); Bermuda Island (*J. bermudiana*) and mainland (*J. silicicola, J. virginiana*).

Principal coordinates 4 and 5 account for only 10.4 and 8.9% of the variance among taxa. Principal coordinate 4 chiefly distinguishes *J. virginiana* (VG) from *J. silicicola* (SI) and principal coordinate 5 separates *J. ekmanii* (EK) from *J. gracilior* (GR). The superimposed minimum spanning tree tends to emphasize the similarity of *J. bermudiana* (BM) and *J. lucayana* (LU).

Discussion

As recently as 1966, Moore [10] designated all the junipers of the West Indies as *J. bermudiana*. Clearly there is much more diversity than he recognized. Florin [3] appears to have had a very good grasp of the diversity. It is apparent from the study of the volatile oils (and leaf morphology, to be reported later) that *J. bermudiana* is quite distinct from other taxa, but closely related to *J. lucayana* from the Bahamas. The former has been threatened by two scale insects [11, 12] and Groves [12] estimated that 90% of the original population discovered on Bermuda were dead by 1955. In 1978, Dr. W. E. Sterrer, Director of the Bermuda Biological Station (personal communication), estimated that perhaps 99% of the original trees were dead. Because of its precarious biological status, it is important to encourage efforts to save this species from extinction.

Juniperus ekmanii and *J. gracilior* are very similar in oil composition which may reflect a common ancestor of relatively recent times. On the basis of volatile oil composition alone, they might well be considered conspecific. However, they do differ considerably in their leaf morphology and formal changes in nomenclature are premature at this time. The same argument applies to *J. silicicola* and *J. virginiana* except these taxa are not only very similar in oil composition but also very similar in morphology and strong consideration must be given to the reduction of *J. silicicola* to a varietal or perhaps populational form of *J. virginiana*. The recognition of *J. lucayana* as a taxon distinct from *J. bermudiana* is supported by the volatile oil analysis.

On the basis of volatile oils, it appears that the migration and evolution of the West Indian junipers may have involved a "stepping stone" phenomenon with migration proceeding from *J. virginiana* to *J. silicicola* on the coastal fore-dunes of the south-eastern United States to *J. lucayana* in the Bahamas, Jamaica, Cuba (and probably Hispaniola). *Juniperus bermudiana* could have arisen by long distance dispersal from the latter. The junipers of Hispaniola are not especially closely related to the other island junipers or to the mainland junipers of the south-eastern United States. Whether they have affinities to the junipers of southern Mexico and Guatemala, which would support the hypothesis of Rosen [13] for contact between a proto-Antilles land plate and the southern Mexico–Guatemala region, is speculative.

Alternatively, *J. bermudiana* could have arisen by long distance dispersal from *J. ekmanii* of Hispaniola, as suggested by the minimum spanning tree (Figs. 1 and 2) and by the similarity of leaf morphology between these two taxa. However, it then becomes difficult to explain the high similarity of oil composition of *J. bermudiana* to *J. lucayana* rather than with *J. ekmanii*.

At present, no nomenclatural changes are proposed for the West Indian junipers except to argue for the recognition of the following taxa: *J. bermudiana*, endemic to Bermuda; *J. ekmanii*, endemic to Hispaniola, *J. gracilior*, endemic to Hispaniola; *J. lucayana*, the only native juniper of the Bahama Islands, also in Jamaica, Cuba and possibly in Haiti (but no extant populations were found in the latter island during field trips of 1979–1980); *J. silicicola* on the coastal fore-dunes in the south-eastern United States.

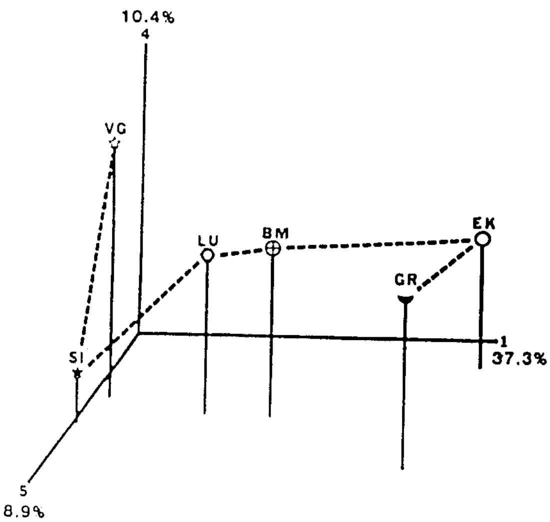

FIG. 2. PRINCIPAL COORDINATE ANALYSIS USING COORDINATES 1, 4 AND 5 WITH A MINIMUM SPANNING TREE SUPERIMPOSED (DASHED LINE). Coordinate 4 separates *J. silicicola* and *J. virginiana*. Coordinate 5 separates *J. ekmanii* from *J. gracilior*.

Experimental

Samples consisted of 10–12 branchlets, 12–15 cm long from the following taxa (acronym, No. of plants sampled): *J. bermudiana* (BM, 15), John Smith's Bay, Bermuda, Adams 2553-2567, 12 December, 1978; *J. ekmanii* (EK, 2), Mare Rouge, Morne de la Selle, Haiti, Adams 3106–3107, 18 February, 1981; *J. gracilior* (GR, 10), Constanza, Dominican Republic, Adams 2785–2794, 3 April, 1980; *J. lucayana* (LB, 10), Pelican Lake, Grand Bahama Island, Adams 2706–2715, 27 March 1980; *J. silicicola* (SI, 10), Oak Hill, Florida, Adams 2775–2784, 31 March 1980; *J. virginiana* L. (VG, 15) near Washington, D.C., Adams 2409–2423, 29 January 1977.

Foliage samples from the Caribbean were frozen locally and then transported by air to the laboratory, where they were kept frozen (−20°) until morphological vouchers were taken and the balance of the foliage steam was distilled to remove the volatile oils [14]. Voucher specimens are on deposit at SRCG.

The volatile terpenoids were analysed by capillary gas/liquid chromatography and peak identifications were based on mass spectral-computer searches [15]. Principal coordinate analysis (PCO) used an F-1 (F from ANOVA) weighted Gower metric [16, 17] following the programmes of Gower [18] and Blackrith and Reyment [19].

In order to assess the affinities of the junipers of the Caribbean and south-eastern United States, ANOVA was performed on six taxa (BM, EK, GR, LU, SI and VG) for the chemical data. The character set generated from this analysis

(F ratios greater than 1.0) included 55 volatile oil characters (Table 1). The chemical data set was then used to compute F-1 weighted Gower similarity measures among the six taxa. This matrix was then factored using PCO. Canonical variate analysis (CVA) was found to give similar results to PCO. However, CVA did not seem applicable since only two samples were available for *J. ekmanii*. Therefore, CVA was discarded in favour of PCO.

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