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The South-western USA and Northern Mexico One-seeded Junipers: their Volatile Oils and Evolution

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Key Word Index – *Juniperus erythrocarpa*; *Juniperus monosperma*; *Juniperus pinchotii*; Cupressaceae; terpenoids, evolution.

Abstract - The composition of the volatile oils of *Juniperus erythrocarpa, J. monosperma, J. monosperma* var. *gracilis* and *J. pinchotii* are reported from analysis by capillary GC MS-computer search. *Juniperus erythrocarpa* appears to have two chemical types or races, one from southern Arizona–south-west New Mexico and the other from Mexico and trans-Pecos Texas. *Juniperus monosperma* var. *gracilis* contained aromatics from the phenyl propanoid pathway marking the first report of these type compounds from the denticulate leaf junipers. *Juniperus monosperma* var. *monosperma* was not found to be similar to *J. monosperma* var. *gracilis*, suggesting a nomenclatural change is needed for the latter taxon. The evolution within this complex has apparently been discordant between the morphology and the terpenoids.

Introduction

The common one-seeded junipers of west Texas, New Mexico, Arizona, and northern Mexico are *Juniperus pinchotii* Sudw., *J. erythrocarpa* Cory, *J. monosperma* (Engelm.) Sarg. and *J. monosperma* var. *gracilis* Mart [1]. These junipers are closely related [1–5] and difficult to distinguish, morphologically [1]. *Juniperus erythrocarpa* in the trans-Pecos Texas region has been lumped with *J. pinchotii* [2, 6] and informally analyzed with *J. pinchotii* populations [3]. In Arizona, Vasek and Scora [7] in a preliminary report on the leaf oil terpenes recognized two chemotypes of *J. monosperma*, "A" and "B". Examination of their specimens revealed that *J. monosperma* "A" is in fact *J. erythrocarpa* while the "B" chemotype is *J. monosperma* itself [1]. In Arizona, *J. monosperma* occurs upon the Mogollon Rim, whereas *J. erythrocarpa* occurs southward in Arizona at lower elevations into Mexico and to west Texas (see [1] for distributions). Some variation in *J. erythrocarpa* was noted by Martinez [8] who recognized *J. erythrocarpa* Cory in the trans-Pecos Texas region but called the taxon *J. erythrocarpa* var. *coahuilensis* Mart. in Coahuila and Chihuahua, Mexico. *Juniperus erythrocarpa* var. *coahuilensis* was not recognized by later workers [1, 4, 5]. Martinez [8] also recognized a taxon from near San Luis Potosi. (La Angostura), Mexico, as having affinities with *J. monosperma* from the USA and named this var. *gracilis* Mart. In the course of populational analyses of the aforementioned taxa, Adams [9] found considerable chemical differences between *J. erythrocarpa* from Arizona and from Mexico and the trans-Pecos Texas region. The purpose of this paper is to report on a thorough identification of the leaf oil components of the taxa as well as the two types of *J. erythrocarpa* and relate the compositions to the evolution of these species.

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Results

Oil yields varied from 3 to 5% dry foliage weight (24 h steam distillation) and in color from clear to light yellow. The components (trace and above) of the volatile oils of the taxa are shown in Table 1. The total number of components per taxon may be misleading since lower attenuations using glass capillaries would undoubtedly reveal many minute trace components beyond the interest of this study. *Juniperus pinchotii* (PIN) and *J. erythrocarpa* from Coahuila, Mexico (ECM) are both high in sabinene, camphor and 4-terpineol. PIN has moderate (3–5%) amounts of limonene, citronellol and bornyl acetate; ECM has moderate amounts of limonene, citronellol, α-pinene and citronellal. A shift is noted in the oil of *J. erythrocarpa* from Benson, Arizona (EBA) with decreased amounts of sabinene and camphor and major amounts of α-pinene, 2 or 4 (2-propenyl)-phenol and an isomer of cuminic aldehyde. EBA has moderate amounts

TABLE 1. COMPOSITION OF THE VOLATILE LEAF OILS OF *JUNIPERUS* SPECIES

Component	% total oil*				
	PIN	ECM	EBA	MG	MON
Tricyclene	t	t	t	-	t
α-Thujene	1.2	0.9	0.6	t	t
α-Pinene	1.7	4.3	8.6	23.9	52.6
α-Fenchene	–	–	–	t	–
Camphene	0.6	t	t	t	0.5
(3-Methyl-4-methylene-(3.2.1)-oct-2-ene) *RRT*=0.204	–	–	1.9	2.0	–
Sabinene	23.4	21.9	7.4	1.2	t
β-Pinene	t	t	0.9	1.0	1.1
Myrcene	2.8	1.7	1.7	2.4	1.7
4-Carene	–	t	–	–	0.7
α-Phellandrene	t	–	–	–	–
3-Carene	–	–	t	10.0	7.8
α-Terpinene	1.6	–	t	t	t
p-Cymene	t	1.7	t	t	t
1:8-Cineole	–	1.2	–	t	–
β-Phellandrene	t	–	t	0.5	0.8
Limonene	3.7	3.5	4.2	1.2	7.3
trans-Ocimene	–	–	t	t	–
γ-Terpene	2.5	2.8	0.7	0.6	1.8
Unknown 1, (-terpineol isomer), *RRT*=0.294	1.4	1.3	t	–	–
(*p*-Menth-1(7),3-diene)	–	–	t	–	–
p-Cymenene	–	–	–	t	–
Terpinolene	1.1	–	t	2.5	–
Unknown 2, terpene alcohol, *RRT*=0.315	–	1.0	–	–	0.6
(β-Terpineol)	1.4	1.9	–	–	–
Unknown 3, terpene alcohol, *RRT*=0.324	–	–	1.4	1.3	–
Linalool	–	t	–	t	–
Unknown 4, terpene alcohol, *RRT*=0.329	–	–	–	–	0.8
cis-Dihydro carveol	0.5	0.7	(t)	(t)	t
Camphor	32.2	14.1	8.7	0.8	0.9
trans-Dihydro carveol	1.2	t	(t)	(t)	–
Camphene hydrate	0.8	0.7	t	t	–
Citronellal	1.3	4.6	(t)	(t)	–
Terpene alcohol, *RRT*=0.368	–	–	–	t	t
Iso-pinocamphone	–	–	t	t	–
Borneol	0.7	0.8	t	t	t
4-Terpineol	7.0	9.9	2.7	t	0.7
Myrtenal	–	–	–	–	t
((3,7,7)-Trimethyl-bicyclo-(3,1,1)-2-heptanone)	–	–	–	1.1	–
α-Terpineol	0.5	t	t	1.0	t
p-Cymenol	–	t	–	–	t
Verbenone	–	–	–	t	t
Myrtenol	–	–	–	–	t
(2 or 4-(2-Propenyl)-phenol), *RRT*=0.420	–	–	6.0	–	–
Cuminic aldehyde isomer *RRT*=0.428	–	–	13.5	1.5	–
Carvone	–	t	–	–	t
Myrtenyl acetate	–	–	1.0	1.6	–
Citronellol	3.4	4.1	–	–	–
Piperitone	–	t	–	t	–
Unknown 5, terpene alcohol, *RRT*=0.459	–	–	2.0	–	t
Bornyl acetate	3.3	1.3	1.0	0.9	0.6
Safrole	–	–	–	1.2	–
Isothymol	–	–	1.1	–	–
Piperitenone	–	–	–	–	0.6
α-Cubebene	–	–	–	t	–
Unknown 6, MW 166? *RRT*=0.557	–	–	0.9	–	–
α-Copaene	–	–	t	–	–
Caryophyllene	t	(t)	t	t	–
α-Cadinene	t	(t)	1.2	0.8	–
Aromatic acetate, *RRT*=0.624	–	–	4.6	t	–
Germacrene isomer, *RRT*=0.640	t	–	1.3	0.9	–
Germacrene D	t	–	3.1	2.0	–
Murolene/cadinene isomer, *RRT*=0.657	t	t	1.8	1.4	–
α-Murolene	(t)	(t)	t	(t)	–
Murolene/cadinene isomer, *RRT*=0.673	t	t	2.0	t	–
γ-Cadinene	0.5	2.0	2.9	3.7	–
δ-Cadinene	t	(t)	2.0	1.6	–
4,10 Dimethyl-4-isopropyl-bicyclo-(4,4.0)-decadiene	t	–	t	(t)	–
Elemol	1.1	2.9	0.8	2.5	2.8
Elemicin	–	–	–	9.6	–
β-Bisabolene	–	–	–	–	t
Cubenol, *RRT*=0.750	t	1.0	2.7	3.1	–
γ-Eudesmol	t	1.1	–	0.7	2.2
Cadinol isomer, *RRT*=0.760	t	(t)	t	0.7	–
Torreyol	–	–	–	t	–
β-Eudesmol	t	1.2	t	0.7	3.7
α-Eudesmol	t	1.2	t	0.9	2.6
Acetate II, *RRT*=0.860	t	0.8	–	t	1.7
Manoyloxide	(t)	–	–	t	–
Total No. of compounds	44	42	52	59	35
No. of unique compounds	1	0	5	7	5

**J. pinchotii* (PIN); *J. erythrocarpa* from Coahuila, Mex. (ECM) and from Benson, Az. (EBA); *J. monosperma* var. *gracilis* (MG) and *J. monosperma* var. *monosperma* (MON). Compound names in parentheses are tentatively identified. Compositional values in parentheses indicate that a component elutes at that retention time but no spectrum was obtained. Trace, t, indicates that the compound was less than 0.5% of the total oil. Components are listed in order of their retention on SP2100. Relative retention times are relative to hexadecyl acetate.

of linonene, an acetate of cuminic alcohol and germacrene-D. Whereas PIN had one unique compound (α-phellandrene, trace) and ECM had none, EBA has five: *p*-menth-1-(7),3-diene, trace; 2-(2-propenyl)-phenol or chavicol, 6.0%; isothymol, 1.1%; unknown #6, *RRT*=0.557, 0.9% and α-copaene, trace. *Juniperus monosperma* var. *gracilis* (MG) from the type locality, has larger amounts of α-pinene, 3-carene, and elemicin with moderate amounts of γ-cadinene, and cubenol. Seven components are unique to MG. These are: α-fenchene, trace; *p*-cymenene, trace; (3,7,7)-trimethyl-bicyclo-(3,1,1)-2-heptanone, 1.1%; safrole, 1.2%, α-cubebene, trace; elemicin, 9.6%; and torreyol, trace. The aromatic methyl ethers derived from the phenyl propanoid pathway form a significant portion of the leaf oil. Safrole and elemicin have been associated with the smooth leaf margined, more mesic junipers (section *Integrae* of Gaussen [11] in North America [10]. The leaf margins of *J. monosperma* var. *gracilis* are quite denticulate. This is the first report of the aromatic methyl ethers in the denticulate section. *Juniperus monosperma* (MON) shows specialization toward the predominance of one compound, α-pinene and two other monoterpenes, 3-carene and limonene as seen in

J. communis [12]. Only one other compound is greater than 3% (β-eudesmol) in MON. The oxygenated fraction of terpenoids is particularly lacking. Five compounds are unique to MON. These are unknown # 4 terpene alcohol, *RRT*= 0.329, 0.8%; myrtenal, trace; myrtenol, trace; piperitenone, 0.6%; and β-bisabolene, trace. Myrtenal and myrtenol, as well as myrtenyl acetate have been found, however, in *J. communis* [12] in the section *Oxycedrus* [11].

Mass spectra for the structurally unknowns (greater than traces) are: compound **1**, *RRT*= 0.294 (*m/e* (%) MW 154(3); 136(10, M - 18), 43(100), 71(78), 93(49), 55(28), 69(27), 81(20), 121(20)), a fast running terpene alcohol, isomeric to β-terpineol; compound **2**, *RRT*=0.315 (*m/e*(%) MW 152(2), 67(100), 41(79), 43(60), 83(56), 109(50), 55(57), 93(57)), a terpene alcohol, either aromatic or with an extra ring (154-2H); compound **3**, *RRT*=0.324 (*m/e*(%) MW 152(1), 43(100), 96(89), 41(78), 109(53), 67(53), 93(47), 79(39), 55(38)), terpene alcohol, either aromatic or with an extra ring (154 - 2H); compound **4**, *RRT*=0.329 (*m/e*(%) MW 152(1), 134(5, M - 18), 43(100), 91(60), 41(54), 109(40), 69(38), 67(32), 82(29)), terpene alcohol, with an extra ring or aromatic; compound **5**, *RRT*=0.459 (*m/e*(%) MW 152(100), 123(53), 91(27), 77(24), 79(20), 109(17), 122(13), 92(13), 66(13)), a terpene alcohol; compound **6**, *RRT*=0.557 (*m/e*(%) MW 166?(20), 151(100), 121(73), 105(39), 91(37), 77(31), 133(29), 149(23), 43(22)); compound **7**, *RRT*=0.860, MW 262 (by chemical ionization), designated as "Acetate II" (see von Rudloff [10,13] for detailed discussion).

Table 2 (upper right) shows the chemical similarities between the taxa. Based on just the presence/absence matching used, *J. pinchotti* (PIN) is about equally similar to both ECM and EBA but the chemical profile (see Table 1) shows that PIN is quite similar to ECM in per cent composition. The differences were chiefly mismatches of trace components. The two *J. erythrocarpa* types are not very similar to each other. *Juniperus pinchotii* (PIN) seems to be linking them. *Juniperus erythrocarpa* from Coahuila, Mexico (ECM) is not very similar to any taxon except PIN whereas *J. erythrocarpa* from Arizona (EBA) has strong affinities to both PIN and *J. monosperma* var. *gracilis* (MG). *Juniperus monosperma* var. *monosperma* (MON) is quite distinct from all taxa with its lowest similarity to *J. monosperma* var. *gracilis*! Similarities based on quantitative matches (Table 2, lower left) show the same trend as presence/absence matching except that *J. pinchotii* is more similar to *J. erythrocarpa* from Coahuila (ECM) than *J. erythrocarpa* from Arizona. *Juniperus monosperma* var. *gracilis* is most similar to *J. erythrocarpa* from Arizona. *J. monosperma* var. *monosperma* shows the same kinds of low similarity as with the presence/absence matching.

TABLE 2. SIMILARITIES BETWEEN POPULATIONS OF ONE-SEEDED JUNIPERS FROM THE SOUTH-WESTERN USA AND NORTHERN MEXICO TAXA

Species*	PIN	ECM	EBA	MG	MON
PIN		0.65	0.66	0.54	0.42
ECM	0.52		0.44	0.41	0.48
EBA	0.41	0.32		0.64	0.35
MG	0.33	0.31	0.47		0.34
MON	0.30	0.36	0.25	0.32	

*Abbreviations are the same as used in Table 1. The similarities of the upper right are based on the number of shared compounds divided by the number of possible matches, excluding trace components where one or both of the values were not confirmed by mass spectral analysis. The similarities of the lower left are based on absolute value differences divided by the range of each component (Manhattan metric) with the exclusion of negative matches and comparisons where one or both of the trace values were not confirmed by mass spectral analysis.

Discussion

Clearly *J. erythrocarpa* is the chemical race "*J. monosperma* A" reported by Vasek and Scora [7]. Detailed examination of populations of *J. monosperma* and *J. erythrocarpa* [9] have revealed *J. monosperma* to be a very uniform species but *J. erythrocarpa* has been found to vary considerably from Arizona to west Texas and thence into Mexico. *Juniperus pinchotii* on the other hand has been shown to be rather uniform, except where it is sympatric with *J. erythrocarpa* in the trans-Pecos Texas region [3]. The chemical similarities suggest that *J. pinchotii* may be an intermediate link between *J. erythrocarpa* from Coahuila, Mexico and *J. erythrocarpa* from Arizona. *Juniperus monosperma* var. *gracilis* is then linked via its chemical similarity to *J. erythrocarpa* from Arizona (EBA). *Juniperus monosperma* var. *monosperma* from the USA is not very similar to any of the other taxa of this study.

Morphologically, *J. monosperma* var. *monosperma* is most similar to *J. erythrocarpa* (from both Arizona and Mexico) and *J. pinchotii* is least similar [1]. No gross morphological differences were observed between northwestern populations of *J. erythrocarpa* from Arizona and those from the trans-Pecos Tex and Mexico areas [1]. The higher chemical similarity between *J. monosperma* var. *gracilis* (MG) and the Arizona portion of *J. erythrocarpa* argues for a closer relationship than previously suggested. Whether *J. monosperma* var. *gracilis* should be given a new species name or included as a variety of *J. erythrocarpa* must await additional populational analyses (in progress), but the

chemical data do indicate that *J. monosperma* var. *gracilis* is indeed a part of the *erythrocarpa–pinchotii* complex. If *J. monosperma* var. *monosperma* arose from a *J. erythrocarpa*-type ancestor, as the morphology suggests, it likely came from the eastern (Coahuilian, ECM) portion of *J. erythrocarpa* where the ecological site differences between the two taxa are less defined [1] than the north-western EBA where the taxa have become quite differentiated in their habitat requirements.

Experimental

Fresh foliage was collected and frozen until steam-distilled from *J. erythrocarpa* on Mex. highway 45, Los Liros, Coahuila, Mexico and south of Benson, Arizona: *J. pinchotii*, Canadian, Texas; and *J. monosperma*, Canadian, Texas; *J. monosperma* var. *gracilis*, La Angostura, San Luis Potosi, Mexico. Voucher specimens are filed at the Science Research Center. The volatile terpenoids were removed by steam distillation for 2 h [14] for quantification analyses and 24 h for yield calculations and the extracts kept at −20° until analyzed to minimize chemical degradation.

GC/MS analyses were run with a Finnigan Model 4000 Quadrapole Gas Chromatograph-Mass Spectrometer (Finnigan Corp., Sunnyvale, CA). Mass spectral scans were taken repetitively from mass 40 to 300, once per sec [15].

Chromatographic separation was achieved using a specially deactivated SP 2100 glass capillary column 0.25 mm i.d. × 30 meters (J & W Scientific, Supelco Inc.). The column was deactivated by injecting 3 μl of 50% triethanolamine in methylene chloride splitless at 210° and held at that temperature for 2 h.

All MS analyses were made in the split mode (30:1 split ratio) using He carrier with an average linear velocity through the column of 21 cm/sec. The column temperature was held at 55° for 6 min after injection and then programmed at 3°/min to 220°, 2 μl of the sample oils were injected after diluting with Et_2O (1:30). Butyl acetate and hexadecyl acetate were added as internal standards. These compounds were chosen as standards because butyl acetate elutes before the most volatile terpenes and the hexadecyl acetate elutes after most terpenes found in these oils. Quantifications were made by peak area integration and summation using FID.

Identifications were made by comparisons of the MS of each component in the oils with MS of known terpenes and searches of spectra from the Finnigan Library (FinnLib) of the U.S. National Bureau of Standards (NBS). Relative retention times (*RRT* hexadecyl acetate = 1.00) were also compared to the *RRT* of known terpenoids run under the same conditions.

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