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Chemosystematic Studies of the Western North American Junipers Based on their Volatile Oils

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Key Word Index – *Juniperus californica*; *J. monosperma*; *J. occidentalis* var. *australis*; *J. occidentalis* var. *occidentalis*; *J. osteosperma*; Cupressaceae; terpenoids; numerical analysis.

Abstract – Six taxa of western North American denticulate leaved junipers were collected and their volatile leaf oils analysed. Two chemical forms of *J. californica* ('A' and 'B') previously reported were found to differ considerably in their concentration of α-pinene, sabinene, β-pinene, camphor and 4-terpineol. However, principal co-ordinate analysis revealed that the chemical forms of *J. californica* were the most similar taxa in the group and considerably more similar than the two varieties of *J. occidentalis* are to each other. This group of junipers appears to consist of two major subgroups: (1) *J. occidentalis*; and (2) *J. californica, J. monosperma, J. osteosperma*; with *J. osteosperma* being closely related to the northern form of *J. californica* (:A.) and *J. monosperma* being most closely related to the southern form of *J. californica* ('B').

Introduction

The junipers of western North America consist of three groups: (a) arid-land, denticulate leaved taxa, *J. californica* Carr., *J. deppeana* Steudl., *J. erythrocarpa* Cory, *J. monosperma* (Engelm.) Sarg., *J. occidentalis* Hook. var. *occidentalis, J. occidentalis* var. *australis* (Vasek) A. & N. Holmgren and *J. osteosperma* (Torr.) Little; (b) a montane mesic, smooth-leaved margined juniper (*J. scopulorum* Sarg.); and (c) an upper montane mesic, acicular-leaved species (*J. communis* L., sect. *Oxycedrus*).

Vasek [1] recognized two subspecies of *J. occidentalis* (subsp. *australis* from southern California, and subsp. *occidentalis* from northern California, Oregon and Washington). Cronquist *et al.* [2] subsequently changed these subspecific categories to varieties to conform with the common usage of that category [3]. The volatile oil compositions of *J. californica, J. monosperma, J. occidentalis* var. *australis, J. occidentalis* var. *occidentalis* and *J. osteosperma* have been reported in an exploratory study based primarily on relative GLC R_ts [4]. Nevertheless, that study did show considerable differences between the infraspecific taxa of *J. occidentalis*; two different chemical profiles for *J. californica* and two different chemical types (A and B) of, presumably, *J. monosperma*. Subsequent terpenoid analysis utilizing glass capillary chromatography/mass spectrometry combined with intensive field work [5] has revealed that the *J. monosperma* 'A' is in fact *J. erythrocarpa* and *J. monosperma* 'B' is typical of that taxon throughout its range. The volatile leaf oil of *J. occidentalis* var. *occidentalis* from Oregon has been recently reported [6] and shown to be high in sabinene, *p*-cymene, γ-terpinene, 4-terpineol and bornyl acetate. The volatile leaf oil of *J. californica* was apparently analysed by Shibamoto and Jennings [7], although the plant specimen was identified only as "California juniper berry leaves collected near Davis, California".

We would like to present a more complete analysis of the oils of *J. californica* ('A' and 'B' of Vasek and Scora [4]), *J. occidentalis* var. *occidentalis* and var. *australis* and *J. osteosperma*. In addition, chemotaxonomic relationships will be examined, including the closely related *J. monosperma*.

Results

The volatile oil composition of *J. occidentalis* var. *occidentalis* is very similar to the previous report

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TABLE 1. COMPOSITION OF THE VOLATILE LEAF OILS OF *JUNIPERUS OCCIDENTALIS* VAR. *OCCIDENTALIS* (OCC OCC); *J. OCCIDENTALIS* VAR. AUSTRALIS (OCC AUS); *J. CALIFORNICA* 'A' (CAL 'A'); *J. CALIFORNICA* 'B' (CAL 'B'); *J. OSTEOSPERMA* (OST) AND *J. MONOSPERMA* (MON).

Component	% total oil					
	OCC OCC	OCC AUS	CAL 'A'	CAL 'B'	OST	MON
Tricyclene	1.0	0.5	t	t	t	t
α-Thujene	0.8	1.1	t	(t)	t	t
α-Pinene	2.5	3.3	17.6	43.8	1.3	52.6
Camphene	0.9	0.8	t	t	t	0.5
(3-Methyl-4-methylene-(3.2.1)-oct-2-ene)						
$RR_t = 0.204$	(t)	0.5	(t)	(t)	(t)	–
Sabinene	11.2	25.3	4.3	1.3	3.9	t
β-Pinene	(t)	t	8.4	1.7	(t)	1.1
Myrcene	1.5	2.4	1.6	1.7	0.7	1.7
4-Carene	–	0.5	t	t	(t)	0.7
α-Phellandrene	0.8	–	–	–	–	–
3-Carene	2.7	5.4	t	t	(t)	7.8
α-Terpinene	t	1.7	1.6	t	0.5	t
p-Cymene	12.0	2.7	t	t	1.1	t
β-Phellandrene	1.0	2.6	t	t	t	0.8
Limonene	2.2	3.0	3.1	3.9	3.1	7.3
trans-Ocimene	(t)	t	(t)	–	(t)	–
γ-Terpinene	1.8	3.0	2.6	0.5	1.0	1.8
β-Terpineol isomer, $RR_t = 0.294$	–	t	t	t	–	–
p-Menth-1(7),3-diene	0.7	t	t	(t)	0.8	–
(*cis*) Linalool oxide	t	–	–	–	(t)	–
p-Cymenene	t	–	–	–	–	–
Terpinolene	0.9	1.2	1.0	0.6	0.5	–
4-Terpinenyl acetate	0.7	t	(t)	(0.5)	–	–
Compound **1**, terpene alcohol, $RR_t = 0.315$	–	–	–	–	–	*:(
β-Terpineol	(t)	t	0.9	(t)	0.9	–
Linalool	t	–	–	–	–	–
Pinene hydrate	1.1	–	–	–	–	–
Compound **2**, terpene alcohol $RR_t = 0.329$	–	–	–	–	–	0.6
	–	–	–	–	–	0.8
cis-Sabinene hydrate	0.6	0.5	–	–	t	–
(*cis*) Dihydro carveol	–	–	(t)	(t)	–	t
Compound **3**, terpene alcohol, $RR_t = 0.339$	–	–	(t)	(t)	1.0	–
Camphor	t	3.4	23.4	5.3	36.7	0.9
Compound **4**, $RR_t = 0.357$	–	–	(t)	t	1.8	–
Pineocampheol	–	1.0	–	–	–	–
trans-Sabinene hydrate	0.9	–	–	–	3.9	–
Camphene hydrate	–	–	1.3	1.1	–	–
Citronella l	–	–	0.9	2.7	–	–
Isoborneol	–	–	t	t	–	t
iso-Pinocamphone	–	–	(t)	t	–	–
Borneol	1.3	2.6	–	–	5.6	t
4-Terpineol	6.6	8.3	6.5	0.8	7.1	0.7
Myrtenal	–	–	–	–	–	t
α-Terpineol	–	t	t	t	0.6	t
p-Cymenol	–	–	–	–	–	t
Verbenone	–	–	t	–	t	t
cis-Piperitol	(t)	t	–	–	–	–
Myrtenol	–	–	(t)	(t)	2.5	t
trans-Piperitol	(t)	t	(t)	t	(t)	–
Isothymol	0.9	(t)	(t)	(t)	–	–
Compound **5**, terpene alcohol $RR_t = 0.437$	–	–	–	–	1.0	–
Carvone	–	–	–	(t)	–	t
Citronellol	–	–	8.0	11.8	–	–
Piperitone	–	0.6	t	(t)	–	–
Isosafrole	–	–	t	1.0	(t)	–
Bornyl acetate	19.0	4.9	0.9	0.9	10.6	0.6
(Piperitenone)	–	–	–	–	–	0.6
Compound **6**, Terpene alcohol $RR_t = 0.512$	(t)	(t)	(t)	(t)	0.8	–
[2,6-Octadienoiacid, 3,7-dimethyl-, methyl ester, (*E*)-]	0.6	t	–	–	–	–
Thymol	0.5	(t)	–	–	–	–
Citronellyl acetate	–	–	(t)	t	(t)	–
Methyl eugenol	–	–	(t)	1.0	–	–
Thujopsene	–	–	0.8	1.3	–	–
Eugenol acetate	0.9	(t)	(t)	(t)	t	–
α-Muurolene	0.8	(t)	–	–	–	–
γ-Cadinene	2.7	2.0	(t)	(t)	–	–
Calamanene	0.6	(t)	–	–	–	–
δ-Cadinene	2.6	2.1	(t)	(t)	(t)	–
Elemicin	(t)	–	0.8	2.3	–	–
Elemol	(t)	(t)	2.1	3.8	0.7	2.8
β-Bisabolene	–	–	–	–	–	t
α-Cadinol isomer, $RR_t = 0.715$	(t)	1.3	–	(t)	(t)	(t)
[2,6,6-Trimethyl-1-cyclohexane-1-yl-(E)-3-butene-2-one]	(t)	0.8	–	–	–	–
γ-Eudesmol	(t)	(t)	t	0.7	(t)	2.2
Cadinol isomer, $RR_t = 0.760$	1.4	1.0	–	–	–	–
β-Eudesmol	(t)	(t)	0.8	1.6	(t)	3.7
Cadinol isomer, $RR_t = 0.769$	1.2	0.7	–	–	–	–
Cadinol isomer, $RR_t = 0.772$	–	0.7	–	–	–	–
α-Eudesmol	–	–	0.8	1.2	(t)	2.6
Farnesol	–	–	1.0	(0.8)	–	–
'acetate II', $RR_t = 0.860$	–	–	–	–	–	1.7

Compound names in parentheses are tentatively identified. Compositional values in parenthesis indicate that a component elutes at that R_t 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 OV1. RR_ts are relative to hexadecyl acetate.

[6], being high in sabinene, *p*-cymene, 4-terpineol, bornyl acetate and the cadinenes. The var. *australis* shows (Table 1) shifts in concentration towards more sabinene, 3-carene, less *p*-cymene, more β-phellandrene and camphor, and much less

bornyl acetate. Otherwise, these two taxa are quite similar in their oil composition. Vasek and Scora [4] showed two profiles for *J. californica* with type 'A' reported as having major amounts of bornyl acetate, sabinol (tent.) and nonyl acetate (tent.). Our analyses did not show large amounts of bornyl acetate (Table 1) nor any sabinol or nonyl acetate. We have found 4-terpineol and camphor to be much larger in the *J. californica* 'A' than the 'B' type. Conversely, *J. californica* 'B' is dominated by α-pinene as previously reported [4]. *Juniperus osteosperma* is low in α-pinene like *J. occidentalis* but, unlike the latter, is dominated by camphor and bornyl acetate.

Mass spectra for the structurally unknown (greater than traces) compounds are: compound **1**, $RR_t = 0.315$, *m/z* (%) 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 **2**, $RR_t = 0.329$ *m/z* (%) MW 152(1), 134[M − 18] (5), 43(100), 91(60), 41(54), 109(40), 69(38), 67(32), 82(29), terpene alcohol, with an extra ring or aromatic; compound **3**, $RR_t = 0.339$, *m/z* (%) MW 152(1), 108(100), 93(68), 41(46), 67(28), 95(26), an aromatic terpene alcohol; compound **4**, $RR_t = 0.357$, *m/z* (%) MW 156(?), 81(100), 96(76), 92(61), 91(54), 95(53), 94(49), terpene alcohol ?; compound **5**, $RR_t = 0.437$, *m/z* (%) MW 152, 109(100), 84(65), 41(53), 55(39), 83(36), 43(29), an aromatic terpene alcohol; compound **6**, $RR_t = 0.512$, *m/z* (%) MW 152, 79(100), 43(65), 91(50), 41(43), 69(30), 92(28), aromatic terpene alcohol.

In order to better visualize the chemical relationships among the taxa, similarities were calculated and principal co-ordinate analysis (PCO) undertaken. The PCO resulted in five eigenroots that accounted for 32.37, 24.22, 18.98, 15.35 and 9.08% of the variation among the six taxa. These data suggested that the taxa cluster into two major groups (Fig. 1): *J. occidentalis* var. *occidentalis* and var. *australis*; and *J. californica, J. monosperma* and *J. osteosperma.* The two forms of *J. californica*, 'A' and 'B' are the most similar of all taxa. *Juniperus osteosperma* is most similar to *J. californica* 'A', the northern form. *Juniperus monosperma* is most similar to *J. californica* 'B', the southern-eastern form. These affinities are seen in the low values of α-pinene in *J. californica* 'A' and *J. osteosperma* and the high values in *J. californica* 'B' and *J. monosperma* (Table 1). The

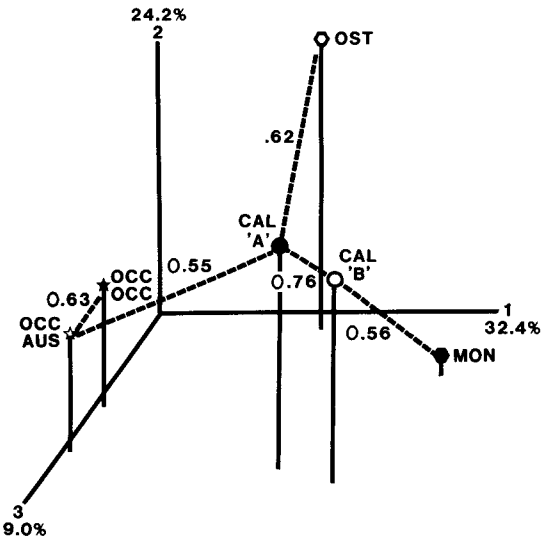

FIG. 1. PRINCIPAL COORDINATE ANALYSIS OF THE SIX TAXA WITH A MINIMUM SPANNING TREE (- - - -) SUPERIMPOSED. The percent variation among taxa explained by each principal coordinate is indicated on each axis. Taxa are: CAL 'A', *J. californica* 'A'; CAL 'B', *J. californica* 'B'; OCC AUS, *J. occidentalis* var. *australis*; OCC OCC, *J.o. occidentalis*; OST, *J. osteosperma*; and MON, *J. monosperma*. Similarities are shown next to (- - - -). Axis 1 separates *J. occidentalis* varieties and axis 2 separates *J. osteosperma* and *J. monosperma* from each other and the other taxa. Axis 3 separates *J. osteosperma* and *J. monosperma* from the other taxa.

same trend, but with *J. californica* 'A' and *J. osteosperma* being high and *J. californica* 'B' and *J. monosperma* being low, is seen in sabinene, α-terpinene, limonene, γ-terpinene, camphor, 4-terpineol, elemol, γ-eudesmol, β-eudesmol and α-eudesmol. It is interesting to note that *J. monosperma* is distributed to the south of *J. osteosperma*. Also of interest is the clustering (Fig. 1) of *J. occidentalis* var. *australis* (the southern variety) with the northern form of *J. californica* ('A').

Axes 4 and 5 removed 15.4% and 9.1%, respectively, of the variance among taxa. Axis 4 primarily differentiates the two varieties of *J. occidentalis* (Fig. 2). Axis 5 primarily separates the two forms of *J. californica* (Fig. 2).

Discussion

Considering the close morphological similarity of these taxa [1], their chemical diversity is considerable. This report confirms the chemical diversity previously reported [4] but lends itself to a somewhat different interpretation. Although ancient or recent hybridization may be involved [4],

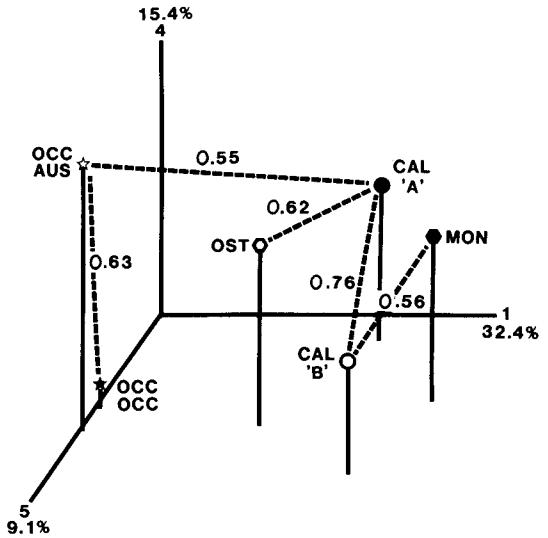

FIG. 2. PRINCIPAL COORDINATE ANALYSIS OF THE SIX TAXA. Definitions are as in Fig. 1. Axis 4 separates the two varieties of *J. occidentalis* and axis 5 separates the two chemical forms of *J. californica*.

the pattern of higher similarities between taxa from adjacent ranges may be due to evolutionary divergence with time. The evolution of the junipers of the western hemisphere is not known, but the center of diversity of the denticulate leaved junipers appears to be in Mexico [3, 8–10]. Even though *J. californica* appears to be graphically central to the diversity of these six taxa (Fig. 1), it is not near the present geographical center of diversity for the arid-land, denticulate leaved junipers.

Vasek [1] argues from the geologic record that *J. occidentalis* var. *occidentalis* is a recent derivative from *J.o. australis.* This hypothetical evolutionary pathway is also supported by the chemical data (Fig. 1). The evolution of *J. occidentalis* var. *occidentalis* from *J. californica* ('A') through *J.o.* var. *australis* may be suggested from the minimum spanning tree while *J. osteosperma* could have evolved from the northern form of *J. californica* 'A' as suggested by the minimum spanning tree (Fig. 1). It seems unlikely that *J. monosperma* evolved from the southern form of *J. californica* ('B') since *J. monosperma* has close similarities to members of the denticulate junipers in Mexico [9, 10]. The apparent link to *J. californica* 'B' may merely be due to the fact that the nearest ancestor of *J. monosperma* was not included in the ordination.

Although the two forms of *J. californica* were consistent among trees from two locations for each type, additional samples would been needed to establish the distribution of these two chemical races. No morphological differences were apparent during field collections; however, more intensive investigation may yet reveal characters that permit recognition of the chemical races. Until such are found, we will treat these as chemotypes of a single morphological entity. The morphological differences used to justify the creation of the two infraspecific taxa of *J. occidentalis* [1] are supported by chemical differences and further substantiate the recognition of these varieties.

Experimental

Samples consisted of 10–12 branchlets, 12–15 cm long from the following: *J. californica* 'A', San Bernardino Mts., Adams 2916–2918, N. Amboy, CA, Adams 2622–2624; *J. californica* 'B', S. Riverside, CA, Adams 2619–2621, S. Yucca, AZ, Adams 2625–2629; *J. monosperma*, Kenton, OK, Adams 2456; *J. occidentalis*, Baker, OR, Adams 1723–1724; *J. osteosperma*, Utah, Adams 1689–1694, 2032–2036, 2168–2172, 1856–1860, 2117–2121. Fresh foliage was frozen until steam distilled. The volatile terpenoids were removed by steam distillation for 2 h [11] for quantification analyses and 24 h for yield calculations. The extracts were kept at −20° until analysed to minimize chemical degradation. Voucher specimens are on deposit at SRCG.

Gas chromatographic–mass spectral analyses were run with a Finnigan Model 4000 Quadrapole Gas Chromatograph–Mass Spectrometer (Finnigan Corp., Sunnyvale, CA). MS scans were taken repetitively from mass 40 to mass 300 every second [12].

Chromatographic separation was achieved using a J & W fused quartz capillary column 0.32 mm i.d. × 30 m coated with OV1 (= DB1). 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/s. The column temp. was held at 55° for 6 min after injection and then programmed at 3°/min to 220°, 2μl of the sample oils was 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 (Finn Lib) of the U.S. National Bureau of Standards (NBS). RR_ts (RR_t hexadecyl acetate = 1.00) were also compared to the RR_t of known terpenoids run under the same conditions.

Similarities between taxa were computed as unweighted Gower metrics [13, 14] with the chemical matches divided by the maximum value encountered in the study. Terpenoids with a range less than 0.8% among the six taxa were not used in the calculation of similarities. This resulted in 44

terpenoids used in the analyses. The similarity matrix was factored by principal coordinate analysis following the programs of Gower [15] and Blackrith and Reyment [16].

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