THE VOLATILE TERPENOIDS OF *JUNIPERUS BLANCOI* AND ITS AFFINITIES WITH OTHER ENTIRE LEAF MARGIN JUNIPERS OF NORTH AMERICA

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ABSTRACT.—The volatile oil compositions of four entire leaf margin species of *Juniperus* are reported as analyzed by glass capillary gc-ms. This is the first report on the composition of *Juniperus blancoi* and includes the most comprehensive analyses of the minor as well as major components of *J. scopulorum*, *J. horizontalis* and *J. virginiana*. *Juniperus blancoi* was found to be most similar to *J. scopulorum* and is postulated to have arisen from the southern Rocky Mountain portion of *J. scopulorum* or some common ancestor. The major components of *J. blancoi* are sabinene, manool, 4-terpineol, acetate II, myrcene, 2-nonanone and elemol.

The junipers of continental North America are composed of 22 species with five species in the entire leaf margined group. This apparently natural group has been recognized by Gaussen (1–2) in the sabinoid junipers of Europe, Asia, and North America as "Integrae section Virginioides," although apparently invalidly published. In our chemo-systematic studies using the volatile oils of *Juniperus* (see 3–5 for numerous references), we have reported on various oils and species encompassing nearly all of the species of continental North America. Of the five entire leaf margin junipers of continental North America (*J. blancoi* Mart., *J. horizontalis* Moench., *J. silicicola* (Small) Bailey, *J. scopulorum* Sarg. and *J. virginiana* L.), only *J. silicicola* has not been examined in detail. Since *J. silicicola*'s distribution is chiefly coastal, and it seems to be either sibling to or conspecific with *J. virginiana*, the absence of that taxon from this study should not affect our general consideration of the high elevation species *J. blancoi*. Zanoni and Adams (6) noted that *J. blancoi* from the mountains of western Mexico has a strong morphological similarity to *J. scopulorum* of the Rocky Mountains in the United States. Furthermore, a numerical taxonomic treatment of the oils of the junipers of Mexico showed *J. blancoi* to be more similar to *J. scopulorum* than any other taxon in Mexico or Guatemala. However, no comparisons were made with any of the other entire leaf margin junipers. The recent development of high resolution glass capillary gc-ms computer systems (7) has prompted us to reexamine the oil of this geographically isolated juniper of Mexico and present a much more complete analysis of its oil along with comprehensive analyses of *J. scopulorum*, *J. virginiana* and *J. horizontalis* (cf. 4,7–9). From the results of these analyses we would, then, like to discuss the affinities and origin of *J. blancoi*.

MATERIALS AND METHODS

PLANT MATERIALS.—Fresh foliage was collected and kept frozen until steam distilled from: *Juniperus scopulorum* (10 trees), Keremeos, B.C., Canada; *J. scopulorum* (15 trees), Durango, Colo.; *J. blancoi*, (15 trees), 0.5 km S. of El Salvador, Estado de Mexico, Mexico; *J. virginiana*

(15 trees) 16 km east of Dulles Airport on I495, Washington, D.C.; *J. virginiana* (4 trees), northwest of Canadina, Texas, on US 70; and *J. horizontalis* (10 plants), bank of Saskatchewan River, Saskatoon, Saskatchewan, Canada. Voucher specimens are deposited at the Science Research Center. The volatile terpenoids were isolated by the steam distillation of approximately 200 grams of foliage for 2 hrs (10) and also 24 hrs for yield calculation (4). All berries (female cones) were carefully removed from the foliage and discarded to avoid unfair comparisons between male and female plants. The two-hour distillation removed about 35% of the volatile oil and gave a slight bias toward the more volatile components (10). The oils were dried over anhydrous sodium sulfate and kept tightly sealed in glass vials with foil lined caps at −20° until analyzed.

Mass spectra were recorded with a Finnigan 4000 quadrapole gcms with a deactivated SP2100 glass capillary column, 0.25 mm ID x 30 m (see 11 for conditions). Quantification was made by FID on a deactivated SP2100 glass capillary column (as above) on a Varian 1860 with nitrogen as a carrier gas at an average linear velocity of 12 cm per sec.; the temperature was programmed as: initial temperature, 70°; then 1.5°/min for 18 min; 2.5°/min for 24 min; 6°/min for 6 min; 4°/min for 6 min; and isothermal at 217° for 6 min. Butyl acetate and hexadecyl acetate were added as internal standards. These compounds were chosen as standards because butyl acetate elutes before the more volatile terpenes, and hexadecyl acetate elutes after most terpenes found in these oils.

Identifications were made by comparisons of the ms of each component in the oils with the ms of known terpenes and searches of spectra from the Finnigan library of the 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.

Similarities between population samples were calculated in two manners. First, similarities were obtained with a simple presence/absence matching coefficient. Second, similarities were obtained with the unweighted, Manhattan metric (3) where the character comparisons were divided by the range of each character. In both cases, negative matches (absence-absence) and comparisons involving trace components where no mass spectra were obtained were excluded.

RESULTS

Due to the infraspecific variability in the volatile oils of *J. scopulorum* and *J. virginiana* (4, 7–9, 12–14), we thought it prudent to report on two wide-spaced populations for each of these taxa in conjunction with the report on *J. blancoi*. *Juniperus blancoi* (table, BLM) has a high amount of sabinene and considerable amounts (5% or more) of 4-terpineol, an unknown (iso-safrole isomer, MW162), acetate II, and manool. It has moderate amounts (2–5%) of myrcene, 2-nonanone, and elemol. Both populations of *J. scopulorum* (SCO, SBC) have major amounts of sabinene, but the population from southwestern British Columbia (SBC) has considerable amounts of aromatic compounds (estragole, safrole, methyl eugenol and elemicin derived from the phenylpropanoid pathway as found previously in other *J. scopulorum* and a few *J. virginiana* populations (4). These compounds are essentially absent in *J. scopulorum* from Durango, Colorado, (SCO), which has considerable amounts of limonene, 4-terpineol, the unknown (MW162; iso-safrole), and acetate II. *Juniperus virginiana* from Washington D.C. (VDC) was found to be low in sabinene and highest in limonene, as previously reported (4). This population had considerable amounts of camphor, the unknown (MW162; iso-safrole), bornyl acetate and safrole, with moderate amounts of linalool, methyl eugenol, elemol, and α-cadinol/τ-muurolol isomer. The population of *J. virginiana* from the Texas Panhandle (VTP) is in a canyon north of the Canadian River and represents the western-most location of that taxon. Unlike the VDC population, the VTP population is high in sabinene like *J. scopulorum* with considerable amounts of 4-terpineol, safrole, and elemol. It has moderate amounts of α-pinene, limonene, γ-terpinene, and methyl eugenol. *Juniperus horizontalis* (HOS) had a major amount of sabinene, considerable amounts of α-cadinol/τ-muurolol isomer, and moderate amounts of α-pinene, myrcene, linonene, 4-terpineol, δ-cadinene, and the cadinol isomer (RRT=0.770).

In this group of closely related taxa few compounds were unique. Neither

SBC, SCO nor VTP contained unique compounds. However, the minor components β-terpineol, the two dihydrocarveol isomers, citronellal and possibly methyl citronellate were found only in the *J. scopulorum* populations. No unique compounds were found in *J. blancoi*, however, the large amount of manool is unusual (table 1). *Juniperus virginiana* from Washington, D.C. (VDC) had 6 unique compounds: 1:8 cineole, (*cis*) linalool oxide, fenchone, camphene hydrate, a mono-terpene alcohol (RRT=0.467), and eugenol. *Juniperus horizontalis* (HOS) contained only two unique components: α-humulene and manoyl oxide. A word of caution should be noted regarding components being absent, however; with the increased sensitivity and resolution of glass capillaries, components previously thought to be missing often appear as traces.

TABLE 1. Composition of the volatile leaf oils of *Juniperus scopulorum* from British Columbia (SBC) and Colorado (SCO), *J. blancoi* from Mexico (BLM), *J. virginiana* from Washington, D.C. (VDC) and the Texas Panhandle (VTP), and *J. horizontalis* from Saskatchewan (HOS).[a]

Component	% total oil					
	SBC	SCO	BLM	VDC	VTP	HOS
tricyclene	—	—	—	t	(t)	t
α-thujene	1.4	0.9	0.9	t	1.2	0.8
α-pinene	2.2	4.8	1.6	1.3	3.2	2.4
camphene	t	t	(t)	t	(t)	t
sabinene	37.2	45.9	44.6	7.6	32.8	48.1
myrcene	t	1.4	2.4	1.2	1.7	3.6
4-carene	—	(t)	t	—	—	t
α-phellandrene	t	(t)	—	—	t	—
α-terpinene	2.0	1.0	1.0	t	1.2	0.6
ρ-cymene	1.6	t	t	t	0.5	t
1:8 cineole	—	—	—	0.7	—	—
β-phellandrene	t	t	t	—	t	t
limonene	1.5	6.0	1.8	19.3	2.8	3.4
trans-ocimene	—	t	t	—	(t)	t
γ-terpinene	3.4	1.6	1.8	0.5	2.0	1.1
(β-terpineol isomer) RRT=0.294	1.3	1.3	0.5	t	—	—
(ρ-menth-1(7)3-diene)	—	—	1.2	t	0.8	1.3
(*cis*) linalool oxide	—	—	—	t	—	—
fenchone	—	—	—	t	—	—
2-nonanone	—	t	2.4	—	—	t
terpinolene	1.3	0.8	1.0	0.5	0.8	0.9
nonanal	—	—	t	t	—	t
(β-terpineol)	1.1	2.0	—	—	—	—
4-terpinenyl acetate	—	—	1.3	t	t	t
linalool	2.0	t	t	2.8	1.2	t
2-nonanol	—	—	0.5	—	—	—
dihydro carveol, isomer	0.7	t	—	—	—	—
cis-sabinene hydrate	—	—	t	t	t	t
camphor	—	(t)	t	5.4	—	(t)
trans-sabinene hydrate	—	—	t	—	t	t
dihydro carveol, isomer	t	t	—	—	—	—
camphene hydrate	—	—	—	t	—	—
citronellal	t	(t)	—	—	—	—
borneol	—	—	—	1.4	—	t
4-terpineol	11.3	5.0	5.8	1.6	5.9	3.4
α-terpineol	t	t	t	t	t	t
estragole	1.2	(t)	—	t	(t)	—
cis-piperitol	t	t	t	(t)	t	(t)
trans-piperitol	t	t	t	(t)	t	—
carvone	—	—	—	t	—	t
citronellol	1.5	t	—	1.5	0.9	0.6

TABLE 1. Continued.

Component	% total oil					
	SBC	SCO	BLM	VDC	VTP	HOS
piperitone	—	—	t	—	—	t
methyl citronellate	0.8	(t)	—	—	—	(t)
terpene alcohol, RRT=0.467	—	—	—	0.6	—	—
(carane hydrate), RRT=0.471	t	(t)	t	t	—	t
(isosafrole, cis/trans), RRT=0.474	3.5	6.2	6.3	5.1	1.8	—
bornyl acetate	t	0.5	0.5	6.7	t	t
safrole	11.1	—	—	11.1	13.8	—
eugenol	—	—	—	t	—	—
methyl eugenol	15.5	—	—	2.9	3.1	—
caryophyllene	t	t	t	t	0.7	t
α-humulene	—	—	—	—	—	t
germacrene, isomer 1	—	—	—	t	(t)	t
germacrene, isomer 2	—	(t)	—	(t)	(t)	t
germacrene, isomer 3	—	(t)	—	t	(t)	t
germacrene D	—	(t)	t	t	t	t
α-muurolene	t	t	t	t	t	1.0
γ-cadinene	t	t	t	t	0.6	0.7
calamanene	—	—	(t)	t	—	t
δ-cadinene	0.6	t	t	0.8	0.8	2.7
elemicin	3.4	(t)	—	1.4	0.5	—
elemol	5.9	3.0	2.8	3.7	6.1	t
sesquiterpene alcohol, RRT=0.715	t	1.2	t	t	t	(t)
α-cadinol/τ-muurolol isomer RRT=0.716	—	—	t	2.5	0.9	11.6
(2,6,6-trimethyl-1-cyclohexane-1-yl-(E)-3-butene-2-one) RRT=0.732	t	t	t	0.7	1.1	1.5
(cubenol)	t	(t)	—	—	—	t
γ-eudesmol	0.5	0.7	t	0.6	1.0	t
α-cadinol/τ-muurolol isomer	(t)	(t)	(t)	t	1.0	1.4
cadinol isomer, RRT=0.761	t	(t)	—	t	—	1.3
β-eudesmol	0.6	0.5	t	0.6	0.9	t
cadinol isomer, RRT=0.0770	t	t	t	1.0	0.8	3.6
α-eudesmol	1.2	t	t	1.0	0.8	—
unknown, RRT=0.824	—	—	—	(t)	—	t
acetate II, RRT=0.862	6.3	5.1	5.3	t	1.6	t
unknown, RRT=0.916	—	—	—	(t)	t	t
manoyl oxide	—	—	—	—	—	t
manool	—	(t)	11.0	—	(t)	t

[a]Compound names in parenthesis are tentatively identified components. Compositional values in parenthesis indicate that a trace component elutes at that retention time but no mass 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.

The volatile oil compositions agree well with the previous reports (7–9), except how many additional minor components have been resolved and identified. Due to the use of all-glass systems (which minimizes rearrangements and decompositions) in the current analyses, it is possible that some of the components previously thought (4) to be rearrangement products (eg. elemol acetate) were not detected. This may also account for the lack of methyl vinyl anisole (4, 8) in the current analyses. However, acetate II (4) was detected, and it could be an artifact from the extraction technique (4).

Pair-wise similarities (table 2, upper right) indicated by a simple presence/absence matching coefficient (enzyme present or active vs. missing or inactive)

show *J. blancoi* to be most similar to the southwestern Colorado *J. scopulorum* (SCO), then to *J. virginiana* from Texas (VTP), and much less similar to the northwestern *J. scopulorum* (SBC). The two *J. scopulorum* populations are most similar to each other (.88). *Juniperus virginiana* (VCD), collected from the region where it is postulated to be the ancestral type (12–14), shows considerable divergence from all the groups with a moderate similarity to the Texas Panhandle *J. virginiana* population (VTP). The Texas Panhandle *J. virginiana* population (VTP) seems rather intermediate between all the taxa, possibly indicating the intermediate nature of this population as has been seen in other areas where *J. scopulorum* and *J. virginiana* are in proximity (4, 14). Whether this intermediacy is due to hybridization or differentiation or whether it reflects past evolutionary pathways is not known.

TABLE 2. Similarities between six populations of *Juniperus*.[a]

	SBC	SCO	BLM	VDC	VTP	HOS
SBC		.88	.53	.56	.62	.45
SCO	.56		.73	.55	.68	.55
BLM	.40	.65		.58	.71	.67
VDC	.31	.35	.40		.68	.64
VTP	.49	.56	.60	.46		.62
HOS	.24	.39	.49	.43	.46	

[a]Population abbreviations are the same as used in table 1. Similarities to the upper right of the diagonal are based on the number of shared compounds divided by the number of possible matches. Similarities to the lower left of the diagonal are based on absolute differences divided by the range of each compound (Manhattan metric). In both cases, negative matches and matches involving one or both trace components for which no mass spectra were obtained were excluded.

Similarities based on quantitative matches reflect a holistic approach and relate to the differences in enzyme kinetics as competition for precursors occurs during terpenoid synthesis. These similarities are shown in table 2 (lower left) and reveal a pattern similar to that based on presence/absence. However, *J. blancoi* (BLM) is now more similar to *J. scopulorum* from Colorado (SCO) than SCO is to *J. scopulorum* from British Columbia (SBC). Undoubtedly this is due to the mutual lack of aromatics in BLM and SCO. The similarity of VTP to SCO and BLM is now equivalent to the similarity between the two *J. scopulorum* populations (SCO and SBC), suggesting a close relationship of the Texas Panhandle *J. virginiana* population to *J. scopulorum*. This is also seen in the lower similarity of VTP to VDC.

Although presence/absence matching and quantitative similarity analyses produced similar results, these two methods are based on two quite different chemical assumptions. A simple presence/absence matching relies on the assumption that each gene produces an enzyme that catalyzes the formation of a particular compound. Quantitative matching is really a comparison of enzyme kinetics (plus, of course, presence/absence of the enzyme). Our experience has been that the presence/absence matching is more valuable between species, and the quantitative comparison is more valuable at the infra-specific level.

Morphologically, *J. blancoi* is more similar to *J. scopulorum* than to *J. virginiana* and *J. horizontalis*. Since *J. scopulorum* has now definitely been found in the Sierra Madre Occidental (15), it is separated by only 500 to 600 kilometers from *J. blancoi*, near Durango, Mexico (6). *Juniperus blancoi* is found in mesic spots along running streams in the mountains of western and central Mexico (6). *Juniperus scopulorum* from Mexico is found in very similar habitats in Coahuila and Chihuahua. With the descent of life zones during the Pleistocene (3, 16, 17) epoch and Tertiary period (18), there has been ample opportunity for dissemination of seeds from the central Rocky Mountains to the northern Sierra Madre Occidental (where *J. scopulorum* is presently found) and southward to where *J. blancoi* is found. Of course, *J. blancoi* could be ancestral to *J. scopulorum* and the migration could have proceeded in the opposite direction. If the Appalachian population (VDC) is ancestral for *J. virginiana*, as has been proposed (13), and is further suggested by the 6 unique components found in VDC of this study, then one would tend to accept the center of radiation for the entire leaf margin junipers as being in Appalachia. Appalachia appears to be an ancient land mass (13) and is geographically central to the entire leaf margin species, especially when one considers the Caribbean entire leaf margin junipers.

The bi-lobed fruit found on *J. blancoi* that distinguishes it from *J. scopulorum* and the other entire leaf margin junipers (6) is not, in fact, unique; it occurs throughout the range of *J. scopulorum* but not with the frequency found in *J. blancoi* (personal observation, RPA). Thus it appears that *J. blancoi* was derived from *J. scopulorum* southern Rocky Mountain populations (or a common ancestor) and through isolation and selection has diverged into the present taxon. Additional population work is needed to resolve the divergence of the British Columbia *J. scopulorum* and the Texas Panhandle *J. virginiana* from their respective parental species.

ACKNOWLEDGMENTS

We would like to thank NSF for continued support of chemosystematic studies in *Juniperus* (grants GB24320, GB37315X, DEB77-22331 to RPA) and to the staff of the National Research Council, Prairie Regional Laboratory.

Received 22 April 1980

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