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THE LEAF OIL TERPENE COMPOSITION OF *JUNIPERUS OCCIDENTALIS**

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Key Word Index—*Juniperus occidentalis*; Cupressaceae; Western juniper; mono-, sesqui- and diterpenes, quantitative variation; chemosystematic aspects.

Abstract The chemical composition of the volatile oil of 10 trees each of two Oregon populations of the western juniper was determined by a computerized GC–MS method. The identity of the major components, sabinene, α-pinene, α- and γ-terpinene, ρ-cymene, limonene, terpinen-4-ol and bornyl acetate was confirmed. In addition, tricyclene, α-thujene, camphene, β-pinene, α- and β-phellandrene, car-3-ene, *trans*-ocimene, linalool oxide, terpinolene, *trans*-sabinene hydrate, camphor, camphene hydrate, borneol, α-terpineol, ρ-cymenol, methyl citronellate, citronellyl acetate, carvacrol, cuminic aldehyde, β-bourbonene, several cadinene and cadinol isomers, elemol, γ-, β- and α-eudesmol, and manoyl oxide were identified. Santene, citronellol, and aromatic ethers of the safrole-eugenol type were not found. Tree-to-tree variability of the relative percentages of these terpenes was fairly large and chemosystematic implications are discussed briefly.

INTRODUCTION

The western juniper, *Juniperus occidentalis* Hook., occurs mainly in central Oregon and California, and in more isolated stands in central Washington, western Idaho and northwestern Nevada [1, 2]. In his treatment of North American junipers, Hall [3] subdivided the section *Sabina* (plants with reduced, scale-like leaves) into those with serrated (teeth-like) and non-serrated (entire) leaf margins. The western juniper has serrated leaf margins and appears to be a fairly uniform species [1]. However, in his more recent study of the distribution and taxonomy of three western junipers, *J. occidentalis*, *J. californica* Carr. and *J. osteosperma* (Torrey) Little, Vasek [4] subdivided this species into two groups, *viz.* subsp. *occidentalis* and subsp. *australis* Vasek. The chemical composition of the volatile leaf oil of all three species was investigated by Vasek *et al.* [5, 6] and significant quantitative differences were recorded between the proposed two subspecies of *J. occidentalis*. However, identification was only tentative and incomplete, and some of the quantitative variation may be due to seasonal differences [6–8]. In our review of the chemosystematic signficance of conifer leaf oil composition [8] we compared the leaf oil of *J. occidentalis* of the dormant season (late fall and winter) with that of three junipers with non-serrated leaf margins, *viz. J. horizontalis* Moench, *J. scopulorum* Sarg. and *J. virginiana* L. (see also von Rudloff [9]). In this exploratory analysis, we found the terpene composition to be much more complex and subsequent identification after separation by preparative GLC met with difficulty owing to mixtures being isolated. Recently, we reported on a computerized GC–MS method that permits quick and reliable on-line identification of known terpenes [10]. We have improved this method by use of a high resolution glass capillary column and succeeded in identifying most of the components of the leaf oil of *J. saltillensis* Hall [11]. This communication describes the results obtained with the leaf oil of ten trees each of two populations of *J. occidentalis* (subsp. *occidentalis*) from central Oregon.

RESULTS AND DISCUSSION

The relative percentage of the identified major terpenes found in the leaf oil of western juniper (means from two different populations near Redmond and Prineville) are listed in Table 1. In addition, most minor (less than 1 %) and trace (less than 0.1 %) components could be identified or characterized by GC–MS. These included β-pinene (RR_t 0.412; bornyl acetate 1.000), car-3-ene (0.487), *trans*-ocimene (0.558), linalool oxide (0.600), *trans*-sabinene hydrate (0.736), camphene hydrate (0.741), cuminic aldehyde (0.896), methyl citronellate (0.960), citronellol acetate (1.123), carvacrol (1.132) (previously found only in the wood of junipers [12], β-bourbonene (1.333) and manoyl oxide (2.064). Santene (0.250), which is present in many conifer leaf oils that contain tricyclene and camphene [8], as well as citronellol, sabinol and aromatic ethers of the safrole–eugenol type (common in *J. scopulorum* and *J. virginiana* [9]) were not found. Ten trace components in the monoterpene range (0.25–1.15) remain to be identified.

The sesquiterpenes presented a problem owing to the similiarity of their spectra [13, 14]. Most of these are of the cadinene–muurolene type. Also, the MS of germacrene isomers and β-sesquiphellandrene are similar to those of the cadinene isomers [13, 14]. In the leaf oil of western juniper at least six hydrocarbons of this type, including α-muurolene (1.375), γ- and δ-cadinene (1.394, 1.410), as well

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* NRCC No. 18201.

Table 1. The major terpene composition of the leaf oil of western juniper

RR_t*	Terpene	Redmond	Prineville
0.318	Tricyclene	0.7	2.3
0.329	α-Thujene	1.2	0.9
0.339	α-Pinene	2.9	1.2
0.361	Camphene	0.2	1.0
0.407	Sabinene	14.2	8.4
0.447	Myrcene	1.8	1.1
0.467	α-Phellandrene	1.1	0.5
0.487	Car-3-ene	0.3	0.4
0.493	α-Terpinene	2.7	1.2
0.501	*p*-Cymene	11.6	6.8
0.513	β-Phellandrene	2.3	1.4
0.517	Limonene	1.7	1.2
0.575	γ-Terpinene	4.4	2.4
0.626	*p*-Cymenene†	0.6	0.2
0.633	Terpinolene	1.1	0.7
0.665	Linalool	0.5	1.0
0.711	Camphor	0.4	0.8
0.787	Borneol	1.8	3.6
0.802	Terpinen-4-ol	9.5	6.5
0.830	α-Terpineol	0.4	0.9
0.838	*p*-Cymenol†	1.1	0.3
0.960	Methyl citronellate†	1.5	0.9
1.000	Bornyl acetate	20.6	38.9
1.453	Elemol	1.5	2.8
	Cadinene isomers‡	5.3	4.9
	Cadinol isomers‡	4.2	4.0
—	Eudesmol isomers‡	2.2	3.8
—	Others and unidentified‡	1.2	1.9

* Relative retention times with respect to bornyl acetate (26.42 min).

† Identified by RR_t and MS only. All others also by ^{1}H NMR and/or IR.

‡ See text.

as the related aromatic hydrocarbons calamenene (1.401) and calacorene (1.432), were present. Of the many sesquiterpene alcohols, three cadinol isomers (1.590, 1.594, 1.609) that are found in, e.g. *J. horizontalis* [9], pines and spruces [8], as well as elemol (1.453), γ-(1.574), β-(1.599) and α-(1.604) eudesmol were identified. The latter 4 alcohols are present in many junipers of the section *Sabina* [8, 9, 11].

From the chemical point of view the difficulty in identifying cadinene–muurolene isomers (and, possibly, also the germacrenes) is a disadvantage. But for chemosystematic comparisons it is adequate to compare components with similar MS and GLC RR_t; e.g. the data for the three cadinol isomers of western juniper agreed very well with those of *J. horizontalis*, *Pinus banksiana* Lamb., etc. Also, since cadinene–muurolene type of sesquiterpenes may be derived from the same precursor [15] it may be of little significance which isomer is present (unless, of course, separate enzymes are involved in their formation). For convenience, the relative percentages of the cadinenes, the cadinols, and the eudesmols were combined in this study (Table 1).

Comparison of the leaf-oil composition of *J. occidentalis* with that of the other North American junipers analyzed thus far [5–11, 16–19] shows that both the typical terpenes of the non-serrated leaf (sabinene, methyl citronellate, elemol, eudesmols) and serrated leaf (camphene, *p*-cymene, terpinen-4-ol, bornyl acetate, camphor) type are present. We have previously drawn attention to the relatively high percentages of *p*-cymene in this species and *J. ashei* [8]. This terpene, and possibly *p*-cymenene and *p*-cymenol, may be biosynthetically linked with α- and γ-terpinene, terpinolene and terpinen-4-ol. The latter group is genetically closely linked in the leaf oil of Douglas fir [20]. Another closely linked group of terpenes is santene, tricycline, camphene, camphene hydrate, camphor, borneol and bornyl acetate [20]. It may be significant that in *J. occidentalis* santene is absent and camphor is a small component, whereas the latter is a major constituent in *J. ashei* [8], *J. californica* and *J. osteosperma* [5, 6]. Unusual minor or trace components in *J. occidentalis* are linalool oxide (?*cis*) and manoyl oxide (also found in *J. saltillensis* [11]), cuminic aldehyde and carvacrol.

Tree-to-tree variability in the relative amounts of terpenes was as large as in *J. scopulorum* and *J. virginiana* [9, 19]. Therefore, in studies of geographical variability and differences between subspecies, etc. 15–20 trees per population should be used [19]. Adams (personal communication) is presently engaged in such studies of the western and southern junipers of North America and this aspect will not be pursued further by us.

EXPERIMENTAL

Branchlets (10–30 cm long) were collected during November 1977 from different sides and heights of 10 trees each west of Redmond and near Prineville, OR, to give *ca* 200 g foliage per tree. The samples were transported and stored in the cold and steam-distilled for 24 hr as described earlier [8, 21]. The quantitative composition was determined on 4 different packed GLC columns, relative percentages being determined by peak area integration and summation with a Hewlett-Packard Model 3352 Data system. The oil samples from each population were combined and analysed by an improved GC–MS method [11], using a deactivated 30 m, 0.25 mm I.D. SP 2100 silicone polymer glass capillary column (J.& W. Scientific). Terpene identifications were made by computer searches of MS using the Finnigan Library of National Bureau of Standards and our own files [10]. Preparative GLC. [21] provided mixed fractions of the major terpenes for ^{1}H NMR and IR spectra comparison.

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