

## The Terpenoids of an Ancestral/Advanced Species Pair of *Juniperus*

ROBERT P. ADAMS\*, ERNST VON RUDLOFF†, THOMAS A. ZANONI‡  
and LAWRENCE HOGGE†

\*Science Research Center, Hardin-Simmons Univ., Gruver, TX 79040, USA;

†Prairie Regional Lab., National Research Council of Canada, Saskatoon, Sask., Canada;

‡New York Botanical Garden, Bronx, NY 10458, USA

**Key Word Index**—*Juniperus ashei*; *Juniperus saltillensis*; Cupressaceae; terpenoids; ancestral.

**Abstract**—The composition of the volatile oil of *Juniperus saltillensis* Hall is compared to that of *J. ashei* Buch., a presumed derived descendant of *J. saltillensis*. The ancestral species (*J. saltillensis*) contained 13 compounds not found in the derived species (*J. ashei*). The derived species' oil was essentially a subset of the ancestral oil with only three components being unique to *J. ashei*. This presents a possible case of evolutionary advancement by specialization and simplification.

### Introduction

In the course of our studies on *Juniperus*, we have found a pair of species which represent both the derived and ancestral forms. Based on morphological and geological arguments, Adams [1] postulated that *Juniperus ashei* Buch. was derived from *J. saltillensis* Hall or an extinct relative. *J. saltillensis* has both its closest chemical and morphological affinities to *J. ashei* and vice versa [1, 2]. In addition, the ancestral (or relictual) populations of *J. ashei* are more similar to *J. saltillensis* than are the more modern populations [1]. The terpenoids of the foliage of *J. ashei* were reported by Von Rudloff [3], but the composition of the foliage oil of *J. saltillensis* has not been examined since that taxon was only recently recognized as a species [4]. The purpose of this paper is to report on the oil of *J. saltillensis* and compare its composition with the derived taxon, *J. ashei*.

### Results

The composition of the leaf oil of *J. saltillensis* is shown along with that of *J. ashei* in Table 1. The structures of most of the trace components of *J. ashei* previously reported as unidentified [3] have now been determined. The two oils are similar: both are high in camphor and low in sesquiterpenes. The components of *J. ashei* are essentially a subset of the components of *J. saltillensis* with only three of 43 compounds unique to *J. ashei*, whereas thirteen compounds are unique to *J. saltillensis*. The

main differences are in the C<sub>10</sub> hydrocarbons. There is a larger accumulation of  $\alpha$ -pinene in *J. saltillensis*, more  $\beta$ -pinene, and the presence of a seven-membered ring compound (3-methyl-4-methylene-bicyclo(3.2.1)oct-2-ene) which is tentatively identified based on a similarity of 809/1000 by the Finnigan Library search (MW 134(33);  $m/e$  91(100), 119(73), 41(43), 77(42), 92(33), 105(26)). The only unknown, non-trace components of *J. saltillensis* were a fast running C<sub>10</sub> alcohol (MW 134(33);  $m/e$  91(100), 109(62), 41(52), 43(52), 67(45), 79(31),  $RR_T=0.330$ ), and 'acetate II' (see Von Rudloff [5] for detailed discussion) whose structure is still unknown (MW 202(5)?;  $m/e$  43(100), 59(46), 107(40), 93(38), 79(36), 119(30),  $RR_T=0.862$ ). In addition, there is one unknown trace component (MW 162(10);  $m/e$  79(100), 91(51), 41(37), 93(30), 77(26), 105(22),  $RR_T=0.473$ ) that has also recently been found in the leaf oil of *J. scopulorum* (unpublished results) and deserves comment because a molecular ion of 162 is not normally found in the mass spectra of monoterpenes or their derivatives. Yet, there is some similarity with the spectrum of myrtenol and the presence of the mass ion peak 147 indicates the loss of a methyl group (162-15) which is common in the terpenes. Approximately 97 and 93% of the total oil of *J. ashei* and *J. saltillensis*, respectively, are accounted for (Table 1). The remaining few percentages are distributed among trace unidentified components.

The oil composition of *J. saltillensis*

TABLE 1. COMPOSITION OF THE VOLATILE OILS OF *JUNIPERUS ASHEI* AND *J. SALTILLENSIS*

Compound	% total oil		Compound	% total oil	
	<i>ashei</i>	<i>saltillensis</i>		<i>ashei</i>	<i>saltillensis</i>
Tricyclene	1.2	t	Iso-pinocampnone	-	t
$\alpha$ -Pinene	1.2	14.6	Borneol	1.7	1.3
Camphene	1.6	0.7	4-Terpineol	t	0.8
(Bicyclo-(3,2,1)-oct-2-ene,3-methyl(4-methylene)	-	1.1	3,7,7-trimethyl bicyclo-(3,1,1)-2-heptanone	-	0.6
Sabinene	-	t	$\alpha$ -Terpineol	t	t
$\beta$ -Pinene	t	1.9	Verbenone	t	t
Myrcene	1.6	2.1	$p$ -Cymenol	t	t
$\alpha$ -Terpinene	t	t	Fenchyl acetate	t	-
$p$ -Cymene	1.3	t	Carveol	t	-
1,8-Cineole	-	4.2	Carvone	t	t
Limonene	5.6	4.0	Piperitone	-	t
<i>Trans</i> -ocimene	-	t	(Carane hydrate)	-	t
$\gamma$ -Terpinene	t	t	Unknown RRT = 0.473	-	t
<i>Cis</i> -linalool oxide	t	(t)	Bornyl acetate	12.2	t
Fenchone	t	t	Thymol	-	t
Terpinolene	t	t	$\alpha$ -Muurolene	-	t
Linalool	0.7	-	Elemol	t	1.4
C <sub>10</sub> -OH, $RR_T = 0.330$	-	1.5	$\gamma$ -Eudesmol	(t)	t
Camphor	68.5	54.1	$\beta$ -Eudesmol	(t)	t
Camphene hydrate	1.3	2.1	$\alpha$ -Eudesmol	(t)	t
Isoborneol	t	t	Acetate II, $RR_T = 0.862$	-	t
			Manoyloxide	t	2.7

Compound names in parentheses are tentatively identified. Compositional values in parentheses indicate that a compound runs at that retention time but no MS was obtained. Trace (t) is set at less than 0.5% of the total oil.

resembles many other juniper species [6, 7] in containing a considerable variety of compounds including the two aforementioned unknowns. *Juniperus ashei* is atypical of *Juniperus* in that most compounds are found in trace amounts, especially the sesquiterpenoids.

### Discussion

If *Juniperus ashei* was indeed derived from *J. saltillensis* [1] or a near relative, then there has been a loss of certain enzymes on the terpenoid pathways, from *J. saltillensis* to *J. ashei* (or the enzyme controlling systems have been modified). This has resulted in a simpler chemical profile in the advanced (derived) species, *J. ashei*. This is similar to the case in the Lemnaceae [8, 9] where various flavonoid pathways were lost (or inactivated?) in the evolutionary more advanced genera and species. This loss could be related to specialization and the rapid radiation of *J. ashei* onto the exposed limestone of the Edwards Plateau of central Texas after the mid-continent seas subsided in early Tertiary times (50–70 million years [10]). Obviously one cannot generalize on the loss of compounds as an index to evolutionary advancement, since this would require ancestors to be more and more complex in their chemical composition (and contain more and more genes). Although the general rule may be

to have more complexity in more evolutionary advanced taxa, the case presented here argues for occasional advancements by simplification and specialization.

### Experimental

Fresh foliage was collected and frozen until steam-distilled from *Juniperus ashei* in Austin, Texas, USA and from *Juniperus saltillensis* south of Saltillo, Coah., Mex. on Rt. 54 near Carneros Pass. Voucher specimens are filed at the Science Research Center and TEX. The volatile terpenoids were removed by steam distillation for 2 h [11] and the extracts kept at  $-20^\circ$  until analysed.

Gas chromatographic-mass spectral analyses were run with a Finnigan Model 4000 Quadrupole Gas Chromatograph-Mass Spectrometer (Finnigan Corp., Sunnydale, California). Mass spectral scans were taken repetitively from mass 40 to mass 300 every second [12].

Chromatographic separation was achieved using a specially deactivated SP 2100 glass capillary column 0.25 mm ID  $\times$  30 m (J & W Scientific, Supelco Inc.). The column was deactivated by injecting 3  $\mu$ l of 50% triethanolamine in methylene chloride (v/v) splitless at  $210^\circ$  and held at that temperature for 2 h.

All analyses were made in the split mode (30:1 split ratio) using helium carrier with an average linear velocity through the column of 21 cm/s. The column temperature was held at  $55^\circ$  for 6 min after injection and then programmed at  $3^\circ/\text{m}$  to  $220^\circ$ . The sample oils (2  $\mu$ l) were injected after diluting with diethyl ether (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.

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 National Bureau of Standards (NBS). Relative retention times ( $RR_t$ , hexadecyl acetate = 1.00) were also compared to the  $RR_t$  of known terpenoids run under the same conditions.

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