GAS–LIQUID CHROMATOGRAPHY OF TERPENES

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PART XI. THE VOLATILE OIL OF THE LEAVES OF JUNIPERUS SCOPULORUM SARG.[1,2]

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ABSTRACT

The neutral leaf oil of Rocky Mountain juniper was analyzed by gas–liquid chromatography. *d*-Sabinene was found to be the major constituent (45.7%) and smaller amounts of *d*-limonene (11.4%), *d*-α-pinene (4.2%), γ-terpinene (1.15%), *p*-cymene (1.4%), *l*-linalool (1.2%), *d*-terpinen-4-ol (2.9%), citronellol (0.2%), *l*-β-elemene (0.2–0.3%), three isomeric cadinenes (2.7%), *l*-elemol (6.0%), and safrole (1.85%) were isolated. α-Thujene, camphene, car-3-ene, myrcene, α-terpinene, terpinolene, thujone, isothujone, methyl citronellate, sabinyl acetate, sabinol, geraniol, α- and δ-cadin*ol*, and *trans*-isoeugenol were tentatively identified. An unidentified acetate (II) (4.7%) was isolated from the oxygenated sesquiterpene fraction and another appears to be present in trace amounts.

The composition of the oils from the leaves of four local ornamental plants was found to differ significantly from that of the wild juniper.

Rocky Mountain juniper (*Juniperus scopulorum* Sarg.) is a small bushy tree found mainly in the mountainous areas of Alberta and British Columbia. Except for ornamental purposes this juniper has not any commercial use. The literature is devoid of any reference as to the chemical composition of the foliage or wood. In part IX of this series the analysis of the volatile oil of the leaves of the savin juniper (*J. sabina* L.) was described (1). This communication deals with a similar analysis of the leaf oil of the Rocky Mountain juniper.

In previous work (2) it was noted that prefractionation by fractional distillation may result in considerable isomerization of some terpenes. Prefractionation by low resolution preparative gas–liquid chromatography (g.l.c.) was employed without apparent rearrangement or decomposition (1, 2), but the yield of low boiling monoterpenes was far from satisfactory. In addition, direct preparative g.l.c. of the total oil does not permit collection of many of the minor constituents. Kugler and Kovats (3) report similar findings and have developed group displacement chromatography on modified silica gel to overcome these difficulties. In the present study silicic acid was similarly pretreated with polyethylene glycol to deactivate catalytic sites. On this adsorbent the oil was prefractionated into hydrocarbons and oxygenated components by elution chromatography without apparent rearrangement, dehydration, or polymerization of the more labile components. These fractions were then further subdivided into mono- and sesqui-terpene fractions by preparative g.l.c. on short columns. These four groups were fractionated further into single components by preparative g.l.c. All major and most minor constituents could thus be isolated and characterized, and several of the trace components were tentatively identified by their relative retention times (r.r.t.). The percentage composition (Table I) was determined by measurement of the area under the peaks obtained in linear temperature-programmed runs (Fig. 1). The components identified by r.r.t. only are shown in parentheses. Kugler and Kovats (3) report an analytical error of 3%. However, Janak (4) has shown that the error may be considerably higher on peaks of small area. In our previous studies the errors recorded appeared to be within the range reported by Janak, except that in isothermal runs the

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TABLE I

Percentage composition of the volatile leaf oil of Rocky Mountain juniper

Peak No.	Compound	R.R.T.*	%† A	%† B	Peak No.	Compound	R.R.T.*	%† A	%† B
1	Unidentified	—	0.15	Trace	23	Unidentified	—	0.3	0.8
2*a*	(α-Thujene)	0.29	1.5	4.8	24	Unidentified	—	0.6	0.6
2*b*	*d*-α-Pinene	0.29	4.2		25*a*	Safrole	0.45	1.85	4.3
3	(Camphene)	0.42	0.15	Trace	25*b*	(Geraniol)	—	Trace	
4	*d*-Sabinene	0.62	45.7	35.2	26	Unidentified	0.51	0.3	0.1
5	(Car-3-ene)	0.73	0.7	0.5	27	Unidentified	—	0.1	0.1
6	(Myrcene)	0.82	1.4	0.95	28	Unidentified	—	0.1	0.3
7	(α-Terpinene)	0.94	0.7	0.8	29	Unidentified	0.64	0.7	3.85
8	*d*-Limonene	1.00	11.4	3.6	30	Unidentified	—	0.15	
9	γ-Terpinene	1.40	1.15	2.1	31	Unidentified	—	0.45	0.65
10	*p*-Cymene	1.63	1.4	0.95	32	Unidentified	0.76	0.85	0.3
11	(Terpinolene)	1.79	0.3	0.8	33	*l*-Elemol	0.84	6.0	14.4
12	(Thujone)	0.64	0.3	Trace	34	(Acetate I)	0.95	0.45	0.5
13	(Isothujone)	0.68	0.5	0.5	35	Unidentified	—	0.15	1.3
14	*l*-Linalool	1.02	1.2	3.2	36	(α-Cadinol or γ-eudesmol)	1.18	0.6	1.6
15	(Methyl citronellate)	1.20	0.3	0.5	37	Unidentified	—	0.3	0.5
16*a*	*d*-Terpinen-4-ol	1.40	2.9	6.4	38	(δ-Cadinol)	1.25	2.7	3.0
16*b*	*l*-Elemene	1.42	0.3		39	(*trans*-Isoeugenol)	1.40	0.15	Trace
17	Unidentified	1.60	0.15	0.5	40	Unidentified	—	0.15	—
18	(Sabinyl acetate)	1.90	0.15	0.1	41	Acetate II	—	4.7	5.1
19*a*	Unidentified C_{15}	—	0.7	0.5	42	Unidentified	—	1.15	—
19*b*	(Sabinol)	2.20							
20	Cadinene I	2.46	0.3	0.2					
21	Unidentified	—	0.45	Trace					
22*a*	Cadinene II	3.30	2.4	0.65					
22*b*	Citronellol	2.95	0.2						

*Relative retention times on a 6 ft × ¼ in. PEG 20M column: (*a*) monoterpene hydrocarbons (limonene = 1.00) at 65 °C; (*b*) oxygenated monoterpenes and sesquiterpene hydrocarbons (camphor = 1.00) at 120 °C; (*c*) oxygenated sesquiterpenes (cedrol = 1.00) at 180 °C.

†(A) From a tree from the Forest Nursery, Indian Head, Saskatchewan; (B) From a tree from the Invermere district, B.C.

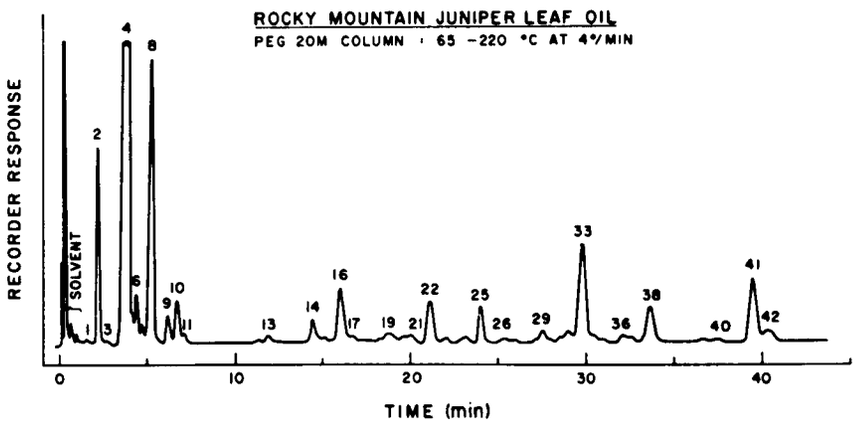

FIG. 1. Gas chromatogram of the volatile oil of Rocky Mountain juniper leaves (6 ft × 3/16 in. o.d. polyethylene glycol 20M column, temperature programmed from 65 to 220 °C at 4° per minute).

values for compounds of high r.r.t. were invariably low. This difficulty was largely overcome by employing temperature programming (1). In the present study an instrument which permitted linear programming was available for the analytical runs and the errors encountered in duplicate runs, or on different columns, were lower than those reported by Janak, but it is unlikely that trace components were recorded with an error of less than 10 to 20%.

Comparison of the composition of the leaf oils from Rocky Mountain and savin junipers shows that both contain *d*-sabinene as a major component and the same monoterpene hydrocarbons, although the limonene content (11.4%) of the former was markedly higher. Common to both oils are also thujone, isothujone, methyl citronellate, terpinen-4-ol, citronellol, geraniol, the cadinene isomers, elemol, and perhaps cadinols. These similarities suggest a close phylogenetic relationship between the two juniper species, but the presence of large amounts of sabinyl acetate and some sabinol in the oil of savin may be a significant difference. Also noteworthy are the relatively high amounts of *l*-elemol, safrole, and the unidentified acetate II (peak 41) in the oil from Rocky Mountain juniper leaves. The latter also contained some *l*-linalool (1.2%) which was not isolated from the oil of savin (the trace component 16 did, however, have the same r.r.t. value).

Since the foliage of the Rocky Mountain juniper was obtained from a prairie location (Indian Head, Saskatchewan), it was possible that the composition of the oil was not typical for this species. Therefore, a small amount of the foliage of Rocky Mountain juniper from the Invermere district, B.C., was steam-distilled and the oil analyzed by linear temperature-programmed g.l.c. No significant qualitative differences were recorded (see Table I, last column) and it is thus probable that the composition of the oil is not markedly influenced by environmental factors. The somewhat lower relative amounts of monoterpenes, especially sabinene and limonene, and correspondingly larger amounts of linalool, terpinen-4-ol and elemene, safrole, component 29 or 30, and elemol are the only noteworthy quantitative differences.

A few garden-variety (ornamental) Rocky Mountain junipers were available locally and small amounts of leaf oils were obtained from four plants. The percentage composition was determined by temperature-programmed g.l.c. and the major components are listed in Table II. Peaks were directly compared with those from the charts of the oils discussed

TABLE II

Percentage composition of the leaf oils of local garden variety Rocky Mountain junipers

		Plant*			
Peak	Compound	C	D	E	F
2	α-Pinene and α-thujene	2.6	2.2	3.1	2.1
4	Sabinene	15.6	6.1	22.5	28.3
8	Limonene	0.5	4.4	0.2	0.6
—	Unidentified	—	—	0.8	Trace
9	γ-Terpinene	1.5	0.4	1.1	0.6
11	Terpinolene	0.3	Trace	0.4	0.3
—	Unidentified	—	—	1.4	0.4
13	Isothujone	2.8	1.6	2.0	0.8
14	Linalool	4.2	3.9	14.1	3.3
15	Methyl citronellate	0.5	Trace	0.5	0.6
16	Terpinen-4-ol	4.4	1.7	4.2	1.6
19	Unidentified	1.5	0.6	0.8	1.0
22	Cadinenes	1.0	0.3	0.6	0.6
—	Unidentified	—	—	2.2	0.4
25	Safrole	0.3	7.0	0.1	0.1
26	Unidentified	0.8	Trace	0.5	0.8
28	Unidentified	1.5	0.3	1.0	0.3
30	Unidentified	0.8	18.6	Trace	0.3
31	Unidentified	9.1	11.0	11.2	3.3
32	Unidentified	1.8	0.9	0.5	3.2
33	Elemol	8.0	4.8	10.6	21.6
38	(δ-Cadinol)	1.8	4.1	1.7	0.7
—	Unidentified	1.8	1.1	0.7	0.8
41	(Acetate II)	28.6	24.1	11.2	20.0
42	Unidentified	1.8	1.5	0.3	0.5

*3–4 years old (commercial seedlings).

above, and some very marked quantitative differences were noticed. Thus, the oil from all four local plants had a lower content of the major monoterpene hydrocarbons, most noticeable being the low content of sabinene in plant D and the virtual absence of limonene in the other three. The content of oxygenated monoterpenes also differed somewhat; e.g. the content of linalool in the oil from plant E was 14.1% as compared with 1–3% in that of the wild trees. No significant differences were found in the sesquiterpene hydrocarbon range, but safrole was found only in trace amounts in plants C, E, and F. The markedly higher content of the components in the oxygenated sesquiterpene range is also noteworthy.

Most noticeable is the fact that the oil from plant F contained over 20% elemol and all four oils had a surprisingly high content of component 41 (acetate II). It is possible that the latter has chemotaxonomic significance, and attempts to isolate sufficient amounts for complete characterization are now being made. The large amounts of component 31 in the oils from plants C, D, and E, and of component 30 in that from plant D, are also noteworthy. Since all leaves were harvested during winter, seasonal variations cannot account for the differences recorded. One possibility is that the differences are due to age, the local plants all being relatively young (3–4 years old). Alternatively, the garden varieties may differ genetically from the wild plants. It follows that for the determination of the taxonomically representative composition of conifer leaf oils, the leaves of mature, wild species should be used.

EXPERIMENTAL

The g.l.c. techniques used have been described previously (1, 2). In addition, a F&M model 500 (F&M Scientific Corp.) chromatograph was used for analytical runs using linear temperature programming. Optical rotations were measured at 25 ± 2 °C of either undiluted material or chloroform solutions (1 to 5%). Infrared spectra were recorded with a Perkin–Elmer model 21 double beam spectrophotometer as films between sodium chloride plates. Individual components were identified by comparison of their infrared spectra and relative retention times (r.r.t.).

Materials

The foliage of Rocky Mountain juniper was obtained from the Indian Head Forest Nursery, Saskatchewan (Plant A). Woody branches and berries were removed and the residual leaves (3.90 kg) were steam-distilled for 3–4 h. The distillate was taken up in ether, washed with bicarbonate solution and water, dried over anhydrous sodium sulphate, and the ether then evaporated at 70–80 °C. The residual oil (58.9 g, 1.51%) had n_D 1.4790; $[\alpha]_D$ +40.3°. Acidification of the bicarbonate solution gave only trace amounts of ether-soluble acids. The foliage (5–10 g) from mature Rocky Mountain juniper from the Invermere district, B.C. (Plant B), and from local garden variety plants (3–4 years old, plants C–F) was steam-distilled and the volatile oil (50–100 mg) was isolated as above. Reference compounds were those obtained previously (1, 2). The liquid phases used in g.l.c. columns were commercial products.

Analytical Chromatograms

Temperature-programmed (linear) chromatograms were obtained with 2–3 μl aliquots of oil using 6 ft × 3/16 in. o.d. polyethylene glycol (PEG 20M) or Apiezon N (A–N) columns (Fig. 1). The percentage composition was determined by measurement of the area under each peak (triangulation method) and the average values from two runs on the PEG 20M column are shown in Table I. The values for those components which were unresolved on this column (e.g. α-thujene and α-pinene) were obtained from runs on other columns.

The r.r.t. values and purity of isolated components were determined on PEG 20M, A–N, polyethylene glycol adipate polyester (APEG), rapeseed oil (RO), and ethylene glycol bis-(propionitrile) (EGPN) columns under the conditions (isothermal) described previously (1, 2). The r.r.t. values of monoterpenes were measured relative to limonene, oxygenated monoterpenes and sesquiterpenes relative to camphor, and oxygenated sesquiterpenes relative to cedrol (see Table I).

Prefractionation

Silicic acid was impregnated with polyethylene glycol (carbowax 20M) as described by Kugler and Kovats (3). Aliquots (1.0 g) of oil of savin (1), oil tansy (2), Rocky Mountain juniper leaf oil, and a synthetic mixture of α-pinene, limonene, *p*-cymene, linalool, citronellol, nerol, fenchyl alcohol, and carvone were dissolved in a little hexane and transferred to the top of a column containing the deactivated silicic acid (20–25 g). Elution with hexane (300 ml) gave a mixture of hydrocarbons which showed no oxygenated terpenes on

g.l.c. analysis. The column was then eluted with methanol (300 ml) and the material eluted showed on g.l.c. analysis less than 2% hydrocarbons and all the oxygenated terpenes previously recorded in the mixtures. Equally good results were obtained when only 10 g modified silicic acid were used, but with 5 g the separation was much poorer.

To test whether any dehydration could be detected, similar runs were carried out with linalool (0.45 g) and *l*-elemol (0.08) alone. In each case, no hydrocarbon peak was recorded and the parent alcohol was recovered unchanged. However, the recovery of elemol was only 85% and it is thus possible that some polymerization does take place.

Rocky Mountain juniper leaf oil (10.63 g) was chromatographed as above on deactivated silicic acid (100 g) to give a hydrocarbon fraction (6.42 g) and the oxygenated terpenes (4.22 g). Aliquots (500 μl) of the hydrocarbon mixture were injected onto a 3 ft × 3/8 in. o.d. SE-30 silicone polymer (25% on Chromosorb W, 40–60 mesh) column, which was programmed (non-linear) from 80 to 150 °C in 20 min. The monoterpenes were eluted within 11 min and collected as a single fraction in an ice-cooled collection flask (5 ml). The sesquiterpenes were collected similarly as a second, unresolved fraction. In the same manner aliquots (100 μl) of the oxygenated terpenes were fractionated into mono- and sesqui-terpenes by use of a 3 ft × 3/8 in. o.d. A–N (20% on Gaschrom P, 60–80 mesh) column, programmed from 100 to 220 °C in 30 min.

Monoterpene Hydrocarbons

Preparative g.l.c. of the monoterpene hydrocarbon fraction (repeated injections of 200 μl aliquots) on a 20 ft × 3/8 in. PEG 20M column programmed from 80 to 160 °C in 50 min resulted in the isolation of components 2, 4, 8, 9, and 10. Component 2 was found to be a mixture of α-pinene and tentatively identified α-thujene (r.r.t. = 0.35 and 0.29 on the A–N and EGPN columns). Component 4, the major constituent of the oil, corresponded in all respects to sabinene; n_D 1.4666; $[\alpha]_D$ +108.0° (*c*, 2.0). The g.l.c. analysis did not reveal the presence of any β-pinene, but up to 2 or 3% of this terpene would not be detectable in sabinene (1). The r.r.t. values and spectral properties showed component 8 to be *d*-limonene; n_D 1.4686; $[\alpha]_D$ +104.6° (*c*, 1.8); component 9: γ-terpinene; n_D 1.4732; and component 10: *p*-cymene. Component 1 could not be isolated and components 3, 5, 6, 7, and 11 were obtained only impure and in small amounts. The r.r.t. values showed the latter to correspond to camphene, car-3-ene, myrcene, α-terpinene, and terpinolene respectively. The infrared spec . of the impure fractions were compatible with these assignments.

Oxygenated Monoterpenes

Aliquots (100 μl) of the mixture of oxygenated monoterpenes were chromatographed on a 6 ft × 3/8 in. PEG 20M column (100 to 210 °C in 35 min). Components 14, 16*a*, and 25*a* were isolated almost pure and were found to correspond to *l*-linalool (n_D 1.4610; $[\alpha]_D$ −12.5°), *d*-terpinen-4-ol (n_D 1.4733; $[\alpha]_D$ +19.7°) and safrole (n_D 1.5214) respectively. Analytical g.l.c. on the A–N column showed that safrole (component 25*a*) could be contaminated by a small amount of geraniol. Component 22*b* was obtained impure (n_D 1.4738; $[\alpha]_D$ +30°). It had the typical smell of citronellol and r.r.t. as well as the infrared spectrum were compatible with this assignment. Components 12, 13, 15, 18, and 19*b* could only be identified tentatively by r.r.t. on the analytical PEG 20M, APEG, A–N, and RO columns (see Table I).

Sesquiterpene Hydrocarbons

Preparative g.l.c. on the 20 f. × 3/8 in. o.d. PEG 20M column (30 μl aliquots, 150 to 205 °C in 50 min) gave components 16*b*, 19*a*, 20, and 22*a* in a fairly pure state. Component 16*b* corresponded to β-elemene, n_D 1.4992, $[\alpha]_D$ −6.0°. Since column chromatography of β-elemol had not given any hydrocarbons (see above), β-elemene is considered to be a genuine component of the oil. Component 19*a* was an unidentified hydrocarbon, n_D 1.5035, $[\alpha]_D$ −8.0°, having r.r.t. values similar to β-caryophyllene, but its infrared spectrum differed somewhat from that of authentic β-caryophyllene (unresolved absorption band at 1377 cm^{-1}; additional bands of medium intensity at 1125, 1050, and 910 cm^{-1}). Component 20 corresponded to the cadinene isomer of low r.r.t. value isolated previously from the oil of savin (1), and found in spruce leaf oils (5, 6); n_D 1.5048; $[\alpha]_D$ −58.4°. Component 22*a* corresponded to cadinene, n_D 1.5057, $[\alpha]_D$ +113.0°, and analytical g.l.c. showed it to be a mixture of γ- and δ-cadinene.

Oxygenated Sesquiterpenes

Aliquots (20 μl) of the last fraction were chromatographed on the 6 ft PEG 20M column (160 to 220 °C in 30 min) and components 33, 36, 38, and 41 could be isolated in a fairly pure state. Component 33 corresponded to *l*-elemol; n_D 1.4993; $[\alpha]_D$ −6.9°. Component 36, n_D 1.5096, $[\alpha]_D$ −13.6°, had the r.r.t. of α-cadinol and γ-eudesmol, but since these two alcohols cannot be distinguished (from one another) by their infrared spectra (7), the identification is uncertain. Component 41 had n_D 1.5000 and $[\alpha]_D$ +147.2° and its infrared spectrum suggested it to be an alcohol containing an acetate group (strong bands at 1730–1720 and 1245 cm^{-1}). When a methanolic solution of this component and sodium methoxide was left overnight the compound appeared to be unchanged. A mixed fraction corresponding to components 33 and 34 was also isolated. As expected, the infrared spectrum showed elemol to be present, but in addition bands at 1735 and 1260–1245 cm^{-1} were recorded. This suggests that component 34 may be another acetate. Component 38 had the retention characteristics and infrared spectrum of δ-cadinol, but seeding with an authentic specimen (m.p. 139–140 °C) failed to induce crystallization.

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