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GAS–LIQUID CHROMATOGRAPHY OF TERPENES

PART IX. THE VOLATILE OIL OF THE LEAVES OF JUNIPERUS SABINA L.[1]

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ABSTRACT

The chemical composition of the volatile oil of the leaves of local savin juniper and of commercial oil of savin was determined by an improved gas chromatographic technique. Both oils contain *d*-sabinyl acetate (38, 37.5%) and *d*-sabinene (30.5, 26%) as the major constituents. Smaller amounts of *d*-α-pinene, *d*-myrcene, *d*-limonene, γ-terpinene, *p*-cymene, *d*-isothujone, *d*-terpinen-4-ol, *d*-sabinol, *d*-citronellol, *d*-cadinene, and *l*-elemol were isolated. Trace amounts of α-thujene, camphene, α-terpinene, 1,8-cineole, terpinolene, thujone, geraniol, a cadinene isomer, and δ-cadinol were tentatively identified. A fraction having the typical pungent odor of juniper leaves was isolated, but it was found to consist mainly of the methyl ester of *d*-citronellic acid. Several unidentified oxygenated sesquiterpenes and a phenol ether were isolated in trace amounts.

Some phylogenetic relationships with other thujone-bearing oils analyzed in this series are discussed.

As part of a systematic study of phylogenetic relationships, the volatile oil of the leaves of *Juniperus sabina* L. and commercial oil of savin have been analyzed by means of the improved technique described in the previous paper of this series (1). Fromm (2) found the main constituent of this oil to be *d*-sabinyl acetate and Semmler (3) obtained *d*-sabinene from the hydrocarbon fraction. Other early reports (4) indicate that minor amounts of α-pinene, α-terpinene, *n*-decanal, dihydrocuminyl alcohol, citronellol, geraniol, and an aldehyde (cuminaldehyde ?) are present. More recently, the oil was examined by fractional distillation by Booth (5), who reported the presence of *d*-sabinene (20%), *d*-sabinyl acetate (40%), and minor constituents (40%), including α-pinene, myrcene, limonene, *p*-cymene, *cis*- and *trans*-thujones, sabinol, terpinen-4-ol, and carvacrol. Bruno (6) identified α-pinene, *d*-sabinene, limonene, and sabinyl acetate by gas–liquid chromatography (GLC).

In contrast, the improved technique used in this study revealed the presence of at least 37 components (see Fig. 1). The average composition, as determined on a variety of columns, is shown in Table I. Little qualitative and quantitative difference was found between the commercial oil and that obtained from local savin juniper plants, except that the sesquiterpene content of the latter was higher. The results obtained confirm that *d*-sabinyl acetate is the major constituent, although an almost equal amount of *d*-sabinene was recorded. Goryaev and Dzhalilov (7) found that the leaf oil of a central Asian savin juniper contained as much as 40% sabinene, which is considerably higher than found in European varieties. GLC analysis of the sabinene isolated in this study did not reveal the presence of any β-pinene, but trace amounts may be present. In the monoterpene hydrocarbon range (peaks 1 to 11) appreciable amounts (2 to 7%) of *d*-α-pinene, *d*-myrcene, *d*-limonene, and γ-terpinene (peaks 2, 5, 7, and 9) as well as smaller amounts (up to 1%) of α-thujene, camphene, α-terpinene, 1,8-cineole, terpinolene, and *p*-cymene (peaks 1, 3, 6, 8, 10, and 11) were recorded.

Amongst the oxygenated monoterpenes thujone, *d*-isothujone, *d*-methyl citronellate, *d*-terpinen-4-ol, *d*-sabinyl acetate, *d*-sabinol, and *d*-citronellol were isolated (peaks 13,

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14, 17, 18, 20, 21, and 23). Peak 25 was tentatively identified as geraniol by comparison of retention data. The trace components 15, 16, and 19 were not identified. The typical pungent juniper odor was associated with peak 17, but methyl citronellate prepared by mild oxidation of citronellal (8) had only the mild and pleasant odor expected for this type of ester. Comparison of the infrared and n.m.r. spectra of the isolated and synthetic materials showed no significant difference. However, the former did have a weak ultraviolet absorption (λ_{max}^{EtOH} approx. 280 and 240–230 mμ), indicating contamination with a small amount of an aromatic compound. No reference of which component is responsible for the typical juniper odor was found in the literature.

The oil of savin was found to contain many sesquiterpenes (peaks 22, 24, 27 to 37) and isolation of individual compounds proved difficult, not only because of the small amounts present (see Table I), but because some components decomposed readily. Also, there was some overlap between some of the sesquiterpene hydrocarbons and oxygenated monoterpenes when the more efficient polyester or polyethylene glycol columns were used. Prefractionation on a silicone polymer (SE-30) column did separate the oxygenated monoterpenes from sesquiterpenes (9), but some decomposition was encountered in this study. Similar results were obtained by column chromatography on silicic acid. By a combination of these techniques peaks 22, 24, 27, 33, and 37 were isolated fairly pure and peaks 35 and 36 were obtained as a mixture. Peak 22 was found to correspond to the cadinene isomer, which is obtained when δ-cadinol is dehydrated with pyridine-modified alumina (10). Since its infrared spectrum showed the presence of a terminal methylene group, this is not δ-cadinene (11). Peak 24 was found to be another *d*-cadinene isomer having a terminal methylene group. This may be identical with the major hydrocarbon found in the oil of cade and could be *d*-γ_1-cadinene. Wallach (12) identified cadinene in the oil of savin by formation of the crystalline dihydrochloride, but did not establish the position of the double bonds.

Amongst the oxygenated sesquiterpenes, *l*-elemol (peak 33) was isolated and δ-cadinol (peak 36) was tentatively identified. Juniperol and γ-eudesmol have the same relative retention times as peak 35, but their presence could not be confirmed. Peak 37 had the same retention time as eudesmol (mixture of α- and β-isomers) and the infrared spectrum showed some similarities with that of β-eudesmol, but did not agree in all respects. Sorm *et al.* (13) reported the presence of elemene, junipene, juniperol, juniper camphor, and α- and δ-cadinol in juniper berry oil. In this study a very small amount of β-elemene was obtained by chromatography on silicic acid, but this compound appeared to be derived rather by dehydration of elemol than from the oil itself. Juniper camphor was not found to be present. An attempt was made to classify some of the sesquiterpenes into cadinene or eudalene types by means of dehydrogenation with selenium or palladium-on-charcoal (14), but this technique failed to give positive results on a milligram scale. This aspect and other possible means of identifying or classifying such small amounts of sesquiterpenes are being studied further.

Comparison of retention times showed that aromatic compounds of the allyl benzene type may be present. Peak 26 has the retention time (on PEG 20M) of anethole and peak 27 has that of safrole. Peak 27 was isolated and appears to be a mixture containing mainly a phenol ether. The infrared spectrum differed from that of safrole and it is possible that the compound is estragole (methyl chavicol). Cuminaldehyde, carvacrol and its methyl ether, eugenol, and isoeugenol were not detected.

In previous papers of this series the analysis of the oils of the leaves of *Thuja occidentalis* L. (15), *Thuja plicata* D. Don (16), and *Tanacetum vulgare* L. (1) was described. These

three oils contain thujone or isothujone as the major constituent. A certain biogenetic relationship between these oils and the oil of savin juniper is discernible in that all contain the thujones and several other compounds having a cyclopropane ring. Common to all four oils are also most of the monoterpene hydrocarbons and terpinen-4-ol. However, little or no taxonomic value may be attached to the latter compounds since it is conceivable that they are formed by a non-specific rearrangement or hydroxylation of a common precursor, such as the carbonium-ion derived from geraniol (geranyl pyrophosphate) as proposed by Ruzicka (17). The oil of savin differs from the other three oils in that it contains sabinyl acetate and sabinene as major constituents and several minor components having a terminal methylene group. Also, certain of the oxygenated monoterpenes, including sabinol, methyl citronellate, citronellol, and perhaps geraniol, are specific constituents of the juniper leaves, whereas, for example, carvone and carvotanacetone (besides major amounts of the thujones) are characteristic of the oil of tansy. Thus, it appears that the formation of at least some of the oxygenated monoterpenes may be subject to genetic control and these constituents may therefore have considerable phylogenetic value. Several authors have attempted to find genetic relationships on the basis of the chemical composition of essential oils (18). However, most of the earlier analyses of volatile oils were incomplete and thus do not lend themselves to critical comparison. Other oils will be analyzed by the gas chromatographic technique to determine the scope of the above conclusions.

EXPERIMENTAL

The same gas chromatographic instruments and procedures as described previously (1) were used. Optical rotations of the oils were measured undiluted at 25±2° and those of individual fractions in chloroform solution (1 to 5%). Infrared spectra were recorded with a Perkin–Elmer model 21 double beam spectrophotometer as films between sodium chloride plates and n.m.r. spectra with a Varian 60 spectrograph, using tetramethyl silane as internal standard.

Materials

The commercial oil (Fritzsche Bros., Inc., New York) had n_D 1.4702, $[\alpha]_D$ +65.0°. Steam distillation of the leaves (240 g) of locally grown savin juniper gave a light yellow oil (11.0 g) having n_D 1.4714, $[\alpha]_D$ +47.7°. Reference compounds were either purified commercial samples or those obtained from other plant sources (1). Cadinene was kindly donated by Messrs. Fritzsche Bros., Inc. and elemol, δ-cadinol, juniperol, juniper camphor, and ledol were gifts from Prof. F. Sorm.

Analytical Chromatograms

Temperature-programmed chromatograms were obtained with 1–5 μl aliquots on 6 ft × 1/4 in. O.D. coiled stainless steel column using polyethylene glycol (Carbowax, PEG 20M) (see Fig. 1), Apiezon N, silicone polymer (SE-30) and adipate polyester (NGA) as liquid phases. *n*-Decane, limonene, camphor, terpinen-4-ol and (or) *n*-decanol were used as internal standards in the quantitative measurements. Area measurement (triangulation method) of the peaks accounted for 98±2% of the oils (1).

Isothermal runs were carried out on the same columns and those containing rapeseed oil and ethylene glycol bis-(propionitrile) (EGPN) as liquid phase. Fractions obtained from preparative runs were analyzed for purity on these columns and the identity of essentially pure fractions was confirmed by comparison of spectral properties and retention data. The average percentage composition, as well as some of the physical data of the isolated components, are shown in Table I. Relative retention times of monoterpene hydrocarbons were measured with respect to limonene, those of oxygenated monoterpenes and sesquiterpene hydrocarbons with respect to camphor, and those of oxygenated sesquiterpenes with respect to cedrol.

Prefractionation

The commercial oil (100 ml) was fractionally distilled *in vacuo* (42 mm Hg; takeoff ratio 1:90) through a Podbielniak spinning band column (100×0.8 cm) to give the following fractions:

I (b.p. 63–74° C, 10.5 ml) monoterpene hydrocarbons,
II (b.p. 74–81° C, 17.0 ml) mainly monoterpene hydrocarbons,
III (b.p. 81–123° C, 12.5 ml) mixture of monoterpenes,
IV (b.p. 123–128° C, 6.0 ml) mixture of oxygenated monoterpenes,
V (b.p. 128–132° C, 18.0 ml) mainly sabinyl acetate.

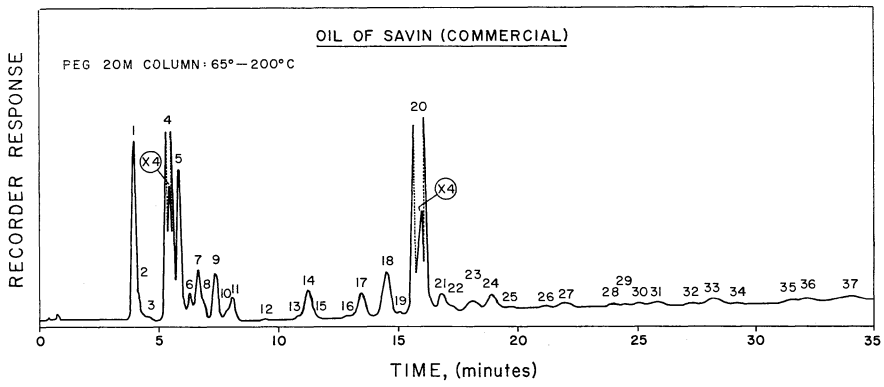

FIG. 1. Gas chromatogram of the oil of savin (6 ft×¼ in. O.D. polyethylene glycol 20M column). (Temperature-programmed from 65 to 200° C.)

TABLE I

Composition of the oil of savin juniper leaves

Peak No.	Compound*	n_D	$[\alpha]_D$	RRT†	Oil from local plants, %	Commercial oil, %
1	α-Pinene	1.4619	+16.9	0.30	2.2	6.8
2	(α-Thujene)	—	—	0.32	0.9	1.0
3	(Camphene)	—	—	0.42	Trace	0.1
4	Sabinene	1.4661	+100.6	0.62	30.5	26.0
5	Myrcene	1.4690	+9.7	0.82	4.5	4.0
6	(α-Terpinene)	—	±0.0	0.94	0.4	1.1
7	Limonene	1.4713	+59.4	*1.00*	1.6	2.7
8	(1,8-Cineole)	—	—	1.07	1.2‡	0.6
9	γ-Terpinene	—	±0.0	1.40	0.7	2.5
10	(Terpinolene)	—	—	1.63	0.7	0.5
11	*p*-Cymene	—	±0.0	1.79	0.4	1.2
12	Unidentified	—	—	0.45	0	Trace
13	(Thujone)	—	—	0.61	Trace	0.5
14	Isothujone	1.4521	+53.3	0.67	0.5	1.9
15	Unidentified	—	—	0.80	0.1	0.2
16	Unidentified	—	—	1.08	Trace	0.2
17	Methyl citronellate	1.4422	+8.1	1.20	1.7	1.8
18	Terpinen-4-ol	1.4762	+22.3	1.40	2.0	3.2
19	Unidentified	—	—	1.60	Trace	0.2
20	Sabinyl acetate	1.4687	+75.9	1.91	38.0	37.5
21	Sabinol	1.4822	+11.1	2.20	0.9	1.1
22	Cadinene isomer	—	—	2.46	0.3	0.5
23	Citronellol	1.4822	+12.0	2.95	1.8	0.8
24	Cadinene	1.5140	+16.5	3.30	4.5	1.5
25	(Geraniol)	—	—	4.85	0.2	Trace
26	(Anethole ?)	—	—	5.40	0.1	Trace
27	(Estragole ?)	1.5100	+3.7	0.40	0.1	0.4
28	Unidentified	—	—	0.45	0.2	Trace
29	Unidentified	—	—	0.55	0.1	Trace
30	Unidentified	—	—	0.58	Trace	Trace
31	Unidentified	—	—	0.61	0.1	Trace
32	Unidentified	—	—	0.66	1.3	0.3
33	Elemol§	1.5070	−9.1	0.84	0.5	0.7
34	Unidentified	—	—	1.05	0.0	0.1
35	(γ-Eudesmol ?)	1.5054	−23.4	1.18	0.8	0.1
36	(δ-Cadinol)			1.25	1.5	0.1
37	(β-Eudesmol ?)	1.5050	−17.2	1.45	2.8	0.2

*Names in parentheses are tentatively identified compounds.
†RRT = relative retention times on 6 ft×¼ in. PEG 20M column: (*a*) monoterpene hydrocarbons at 65° C (limonene = 1.00), (*b*) oxygenated monoterpenes and sesquiterpene hydrocarbons at 120° C (camphor = 1.00), (*c*) oxygenated sesquiterpenes at 180° C (cedrol = 1.00).
‡Could contain another component.
§Melting point 44–50° C.

At this stage some decomposition appeared to take place, and the vacuum was reduced to 10 mm Hg. Distillation was continued rapidly (takeoff ratio 1:20) to give:

VI (b.p. 95–105° C, 20 ml) sabinyl acetate and higher boiling terpenes,

VII (b.p. 105–145° C, 8 ml) mainly sesquiterpenes.

The distillation residue (6–7 ml) was a black tar which was not further investigated.

The oil from the local plants was prefractionated by preparative GLC (3/8 in. O.D. SE-30 column (1)) and fractions of approximately the same composition were collected by operating the collector manually.

Monoterpene Hydrocarbons

Preparative GLC of fraction I on a 6 ft × 3/8 in. O.D. PEG 20M column (30–50 μl aliquots, repeated injection, 65–90° C in 12 minutes) gave two fractions. The first was impure *d*-α-pinene (peak 1), which contained α-thujene (peak 2). The second was essentially pure *d*-sabinene (peak 4). Analytical runs showed no signs of β-pinene, although up to 2% would not be detectable in the sabinene fraction because of the close retention times.

Similar fractionation of fraction II gave *d*-sabinene, *d*-myrcene (peak 5), impure α-terpinene (peak 6), impure limonene (peak 7), γ-terpinene (peak 9), and impure *p*-cymene. Peaks 3, 8, and 10 were tentatively identified by retention characteristics as camphene, 1,8-cineole, and terpinolene respectively.

Almost identical results were obtained with corresponding fractions from the oil obtained from local plants.

Oxygenated Monoterpenes

Fraction IV was fractionated on a 20 ft × 3/8 in. O.D. PEG 20M column (60 μl aliquots, 170° to 200° C in 25 minutes). Peaks 14, 17, 18, and 20 were isolated practically pure and found to be *d*-isothujone, an unknown ester, *d*-terpinen-4-ol, and *d*-sabinyl acetate (see below) respectively. Peak 13 was obtained only impure but GLC and infrared spectra confirmed that it contained mainly thujone.

Methyl Citronellate

The unknown ester (peak 17) had the typical pungent smell of juniper leaves. Strong infrared absorption bands were recorded at 2970–2860, 1735, 1440, 1380, 1362, 1195, and 1153 cm^{-1}, and medium intensity bands at 1010 and 827 cm^{-1}. Saponification confirmed it to be an ester. Found: C, 71.69; H, 10.97; COOH, 23.38%. Calculated for $C_{11}H_{20}O_2$: C, 71.69; H, 10.94; COOH, 24.43%. The n.m.r. spectrum (trimethyl silane = 0.00) contained the following:

Band	Protons	δ	Assignment
Triplet	1	5.18	$\diagdown$C=CH— $\diagup$
Singlet	3	3.67	—OCH_3
Complex	3	2.20–2.00	—
Singlet	2	1.92	—
Doublet	6	1.65	$\diagdown$C=C$(CH_3)_2$ $\diagup$
Complex	2	1.50–1.10	—
Doublet	3	0.95	—C—CH_3 $\diagup$

The data were those expected for methyl citronellate, or a closely related compound. Citronellic acid was synthesized by shaking citronellal with a suspension of silver oxide in very dilute aqueous ammonium hydroxide for 5 hours (8). The synthetic acid was converted to the methyl ester with diazomethane and purified by GLC. The synthetic ester had identical infrared and n.m.r. spectra as well as retention characteristics as those of peak 17. However, the smell was only the faint pleasant odor of an unsaturated aliphatic ester. The ultraviolet spectrum of peak 17 showed only very weak, broad absorption bands near 280, 240, and 230 mμ, indicating that a very small amount of an aromatic aldehyde may be present. This impurity could not be detected by GLC.

Sabinyl Acetate

Fraction V contained better than 95% of peak 20. The infrared spectrum suggested this to be an acetate (1737, 1242 cm^{-1}). Saponification gave essentially pure *d*-sabinol, n_D 1.4868, $[\alpha]_D$ +11.2°, *p*-nitrobenzoate m.p. 75–76° C. The ester was obtained almost pure (cf. Table I) by preparative GLC from fractions IV, V, and VI.

From fraction V of the oil from the leaves of the local plants, pure sabinyl acetate, with n_D 1.4675, $[\alpha]_D$ +78.9°, was isolated.

Higher Oxygenated Monoterpenes

Fraction VI was found to be a mixture of *d*-sabinyl acetate, higher boiling monoterpene alcohols, and

sesquiterpene hydrocarbons. Preparative GLC gave peaks 21, 23, and 24 fairly pure and these were found to be *d*-sabinol, *d*-citronellol, and *d*-cadinene (see below) respectively. Peak 22 appeared to be a cadinene isomer but it was not obtained pure by this technique. The concentration of peaks 25 and 26 was still very low in either fraction VI or VII and could not be isolated in sufficient amounts to record satisfactory infrared spectra.

Sesquiterpenes

To obtain larger amounts of the two cadinene isomers (peaks 22 and 24, see above), aliquots (1.0 g) of fractions VI and VII were chromatographed on silicic acid (30 g). Elution with hexane gave a hydrocarbon fraction which was separated further into three components by preparative GLC. The first was found to have the same infrared spectrum as β-elemene (RRT 1.30). The latter was prepared in small amounts by dehydration of elemol with the pyridine-modified alumina reagent (10). The second component (peak 22) had a similar infrared spectrum and the same retention times as the major hydrocarbon obtained on dehydration of δ-cadinol with the alumina reagent. Typical absorption bands for terminal methylene groups (3070, 1645, and 880 cm^{-1}) were recorded. The third component (peak 24) was obtained in comparatively large amounts; n_D 1.5072, $[\alpha]_D$ +41.6°, infrared bands at 3080, 1645 cm^{-1} (weak), 883, 830, 798 cm^{-1} (medium intensity). It had the same retention times as the major hydrocarbon of the oil of cade.

Elution of the silicic acid chromatogram with chloroform gave a complex mixture of oxygenated mono- and sesqui-terpenes, which was not resolved by preparative GLC. Several decomposition peaks were recorded.

Preparative GLC (6 ft × 3/8 in. O.D. PEG 20M, 115 to 220° C in 30 minutes) of fraction VII gave fairly pure sabinyl acetate (peak 20), *d*-cadinene (peak 24), and peaks 27, 33, and 37; peaks 35 and 36 were collected as one fraction. Peak 27 had a strong ultraviolet absorption (λ_{max}^{EtOH} 276, 235–205 mμ) but no characteristic odor. The aromatic character was confirmed by a sharp, strong band at 1516 cm^{-1} in the infrared spectrum. In addition, the latter showed strong bands at 2930, 1465, 1260, 1235, 1153, 1140, and 1030 cm^{-1}, and medium bands at 3520, 3070, 1725, 1700, 1633, 1605, 1592, 907, and 800 cm^{-1}. Peak 33 had an infrared spectrum which was very similar to that of elemol. The viscous material crystallized on seeding with elemol; m.p. 44–50° C, $[\alpha]_D$ −9.7°. The mixture of peaks 35 and 36 had n_D 1.5054, $[\alpha]_D$ −23.4°, and strong infrared bands at 3440, 2940, 1460, 1375, 1130, 906, and 885 cm^{-1}, and weaker ones at 3090, 1640, 1592, 1505, 1260, 1240, and 800 cm^{-1}. Superimposition of the infrared spectrum of δ-cadinol showed that this compound could be present whereas that of juniperol differed extensively. The infrared spectrum of peak 37 had bands at 3500, 3090 (weak), 2930, 2860, 1710 (weak), 1642, 1592 (weak), 1470–1440, 1385–1370, 1260, 1120 (broad), 1030, 1010, 950 (weak), 932, 910, 885, 875 (weak), 812, and 798 cm^{-1}, and resembled that of β-eudesmol.

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