A STUDY OF THE SUBSTANCES PRESENT IN ESSENTIAL OILS

L. The Composition of the Essential Oil of Juniperus Pseudosabina

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Because of their special properties, the essential oils of the genus Juniperus are widely used in medicine and in the food industry. Thus, the cedrol fraction of J. semiglobosa Rgl. and J. seravschanica Kom. are used for the treatment of supurative and poorly healing wounds and ulcers and also in osteomyelitides [1]. The whole oils of these plants are used for the perfuming of high-quality tobaccos and soaps, and for the preparation of perfumes and toothpastes.

J. pseudosabina Fisch. et Mey (Turkestan juniper) which grows in Kazakhstan (Altai, Tarbagatai, Dzhungarsk Ala-Tau) and in Siberia (Sayany, Transbaikalia, Maritime Territory) is a semidecumbent shrub with a grayish-green acerose leaf and large brownish-black oval fruit [2].

The essential oil of the Turkestan juniper was separated preliminarily by high-vacuum fractionation and by chromatography on alumina. From the fractions obtained, substances of individual composition were obtained by gas-liquid chromatography.

The components isolated were identified by comparing their physicochemical properties and IR spectra with those given in the literature and, where necessary, by the performance of suitable qualitative reactions and the preparation of characteristic derivatives.

The amounts of the substances in the whole oil and in its fractions were determined by the normalization and internal standard method, suitable freshly-isolated terpenoids absent from the essential oil being used as the internal standard.

The gas-liquid chromatography was carried out on a UKh-1 instrument and the IR spectra were recorded on a UR-20 spectrometer.

EXPERIMENTAL

The essential oil, obtained in our laboratory by the steam-distillation of freshly-cut small flowers of the fruit-bearing juniper (collected in the Panfilov region of the Alma-Ata region) had the following constants: d_{20}^{20} 0.8696, n_D^{20} 1.4695, $[\alpha]_D^{20}$ −35.7°, acid no. 0, ester no. 13.5, yield 1.85%.

For the concentration of the individual components in the fractions, the terpene part of the essential oil was distilled off under vacuum through a Widmer column. Distillation was continued until the appearance of oxygen-containing compounds as determined by gas-liquid chromatography and by IR spectroscopy in the 1600–1800 and 3400–3650 cm^{-1} regions. The results of the distillation of the essential oil (1487 g) are given below:

Fraction	Bp (pressure 40 mm Hg)	Wt. of fraction, g
I	66.6–66.8	599
II	66.8–68.4	242
III	68.4–72	264
IV	72 –74.1	59
V	78 –88	13.2
High-boiling fraction (still residue)	—	307

Analysis of the fractions showed that fraction V included a small amount of oxygen-containing substances, and it was therefore added to the high-boiling fraction. The high-boiling fraction, on storage in the cold, gave large colorless crystals with mp 86.5° C (methanol) giving no depression of the melting point with cedrol [3].

To isolate the bulk of the cedrol, the high-boiling fraction (320 g) was diluted with an equal amount of methanol. The cedrol that deposited (110 g) was separated off and recrystallized from methanol. The remainder of the essential oil, present in the mother liquors, was washed free from methanol with water and, after drying, was chromatographed on alumina (activity II–III, ratios 1 : 60 and 1 : 100). This led to a complete separation of the hydrocarbons from the oxygen-containing fraction of the essential oil.

In this way the essential oil was separated preliminarily into monoterpene and sesquiterpene hydrocarbons cedrol, and an oxygen-containing fraction.

The terpene hydrocarbons. A gas-liquid chromatogram of the terpene hydrocarbons of the essential oil (stainless steel column 505 cm long and 0.4 cm in diameter; stationary phase—polyethyleneglycol-1000, 15% of the weight of the solid carrier—INZ-600, 0.25–0.5 mm; rate of flow of hydrogen 70 ml/min; temperature 90° C) showed 12 peaks.

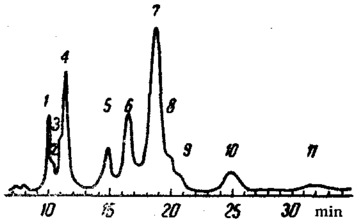

Fig. 1. Chromatogram of the sesquiterpene hydrocarbons of the essential oil of the Tunkestan juniper: 1) α-cedrene; 3) β-elemene; 4) β-cedrene; 2) γ-cadinene; 8) ar-curcumene; 10) calamenene; 11) calacorene.

The terpene hydrocarbons were identified by their preparative isolation from the appropriate fractions on 1.0 × 450 cm columns containing 25% of polyethyleneglycol-2000 or 25% of polyethylene glycol adipate. The results of a comparison of the properties of the components obtained and their IR spectra with those reported in the literature [4] permit the conclusion that the following compounds were eluted successively from the column: 1-α-pinene, d-camphene, 1-β-pinene, d-sabinene, Δ^3-carene, β-myrcene, 1-α-phellandrene, 1-limonene, 1-β-phellandrene, γ-terpinene, p-cymene, and terpinolene.

Sesquiterpene hydrocarbons. A gas-liquid chromatogram of the sesquiterpene hydrocarbons (copper column 3.8 m long and 0.4 cm in diameter); stationary phase PEG-1000, 15% on Celite 545, 60–80 mesh; temperature 180° C; rate of flow of hydrogen 55 ml/min) had 11 peaks (Fig. 1). The components to which these peaks corresponded were isolated on columns (3.80 × 0.7 cm) containing the same phases as were used in the isolation of the terpene hydrocarbons, but the sesquiterpene hydrocarbons were first separated into a number of fractions by vacuum distillation. The physicochemical properties of the sesquiterpene hydrocarbons isolated from the essential oil of the Turkestan juniper are given in Table 1.

A comparison of the properties of the components isolated and their IR spectra with literature data [4,5] permits the conclusion that peaks 1, 3, 4, 7, 8, 10, and 11 belong, respectively, to *l*-α-cedrene, *l*-β-elemene, *l*-β-cedrene, *l*-γ-cadinene, d-ar-curcumene, *l*-calamenene, and d-calacorene.

Oxygen-containing fraction. The chromatogram of this fraction of the oil (Fig. 2) was obtained on a copper column (245 × 0.4 cm) filled with polyethyleneglycol-2000 (15%) on Celite 535, 60–80 mesh, at a column temperature of 160° C with H_2 as the carrier gas at 60 ml/min.

After the saponification of a small sample of this fraction of the oil, peaks 2, 8, 10, 15, 16, and 18 on the chromatogram had disappeared and there was a relative increase in the areas under peaks 1, 12, 13, and 14. This shows that the chromatographic peaks that had disappeared, corresponded to esters of alcohols present in the oil in the free state.

To ensure the isolation and identification of the components, two fractions not containing sesquiterpene alcohols were separated by vacuum distillation.

Table 1

No. of the peak on the chromatogram (Fig. 1)	d_{20}^{20}	n_D^{20}	$[\alpha]_D^{20}$, deg	Component identified
1	0.9298	1.4941	—67	α-Cedrene
2	—	1.4922	—	—
3	0.9004	1.4981	—8.9	β-Elemene
4	—	1.5026	—	β-Cedrene
5	0.9139	1.5048	0	—
6	0.9036	1.4992	—49.6	—
7	0.9173	1.5076	+63.4	γ-Cadinene
8	0.8830	1.5036	+39	ar-Curcumene
9	0.9267	1.5049	—23	—
10	0.9321	1.5195	—34	Calamenene
11	0.9526	1.5404	+31	Calacorene

Table 2

No. of the peak on the chromatogram (Fig. 2)	n_D^{20}	Mp, °C	d_{20}^{20}	$[\alpha]_D^{20}$, deg	Component identified
2*	1.4303	—	—	—	—
3	1.4239	—	0.8267	0	Methyl n-nonyl ketone
4	1.4558	—	0.9141	+28	Thujone
6	1.4653	—	0.8646	—9.8	Linalool
8	—	175—177	—	—	Camphor
9	1.4794	—	0.9351	+17.3	Terpinen-4-ol
11	1.4803	—	0.9176	+67	α-Terpineol
12	—	205—208	—	—	Borneol
13	1.4746	—	—	0	Nerol
14	1.4793	—	—	0	Geraniol
19	—	85—86.5	—	+7.5 (ethanol)	Cedrol
20	1.4941	—	—	—	β-Elemol
21	—	72—74°	—	—	Eudesmol

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*The other substances were identified by comparing their retention times with the retention times of known substances.

The properties of the oxygen-containing compounds obtained from the essential oil of the Turkestan juniper are given in Table 2.

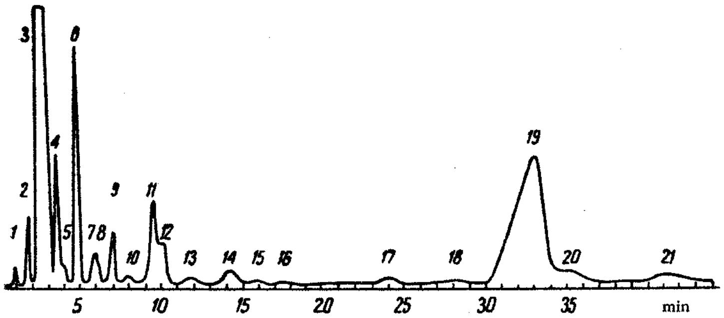

Fig. 2. Chromatogram of the oxygen-containing compounds of the essential oil of the Turkestan juniper: 3) methyl n-nonyl ketone; 4) thujone; 6) linalool; 7) linalyl acetate; 18) camphor; 9) terpinen-4-ol; 10) bornyl acetate; 11) α-terpineol; 12) borneol; 13) nerol; 14) geraniol; 15) neryl acetate; 17) geranyl acetate; 19) cedrol; 20) elemol; 21) eudesmol.

The substance responsible for peak 2 is, according to the IR spectrum (Fig. 3,1) an ester (bands at 1738 and 1180 cm^{-1}) the molecule of which has a methylenic double bond (bands at 896, 1653, and 3081 cm^{-1}). It possesses a strong pleasant odor. Unfortunately, because of the small amount of this substance in the essential oil we were unable to establish its nature. The third substance eluted was a ketone (Fig. 3,2; band at 1713 cm^{-1}, absence of a band at 2720 cm^{-1}), the 2,4-dinitrophenylhydrazone of which had mp 58–60° C.

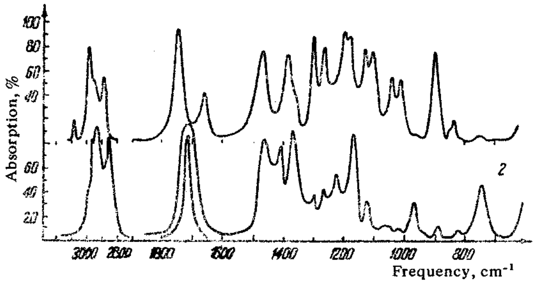

Fig. 3. IR spectra of the ester (1) (peak 2, Fig. 2), and of methyl n-nonyl ketone (2) (IKS-14 spectrophotometer, layer thickness 0.010 mm).

The analytical results permit the assumption that this is an aliphatic ketone of nonterpene nature.

Found, %: C 78.02; H 9.44. Calculated for $C_{11}H_{22}O$, %: C 77.73; H 9.40.

The properties of the substance isolated are similar to those of methyl n-nonyl ketone and propyl heptyl ketone [6,4]. The melting point of the semicarbazone of our substance is 123–123.5° C, which is very close to that of the semicarbazone of methyl nonyl ketone (118° C [4], 123–124° C [7]) and differs markedly from the melting point of the semicarbazone of propyl heptyl ketone (54–56° C [4]).

The results of a comparison of the properties (see Table 1) and IR spectra of the other oxygen-containing compounds isolated with literature data has shown that the essential oil contains d-thujone, *l*-linalool (peak 6), *l*-camphor, d-terpinen-4-ol, bornyl acetate, d-α-terpineol *l*-borneol, nerol, geraniol (peaks 8–13, respectively), d-cedrol (peak 19), *l*-elemol (peak 20), and eudesmol (peak 21) (see Fig. 2).

Thus, the essential oil of the Turkestan juniper contains not less than 43 components and may be regarded as a raw material for the production of highly active levorotatory α-pinene and cedrol. The latter is used for the synthesis of cedrenyl acetate [8] and cedrenecarboxylic acids [9], which are valuable in practice. The high-boiling fraction of the essential oil possesses a good perfume odor and can be used as a fixture in various perfumery products.

CONCLUSIONS

The essential oil of Juniperus pseudosabina Fisch. et Mey, obtained from small flowers by steam distillation has been investigated. It has been established that it contains not less than 43 components, of wich the following have been identified: *l*-α-pinene (67%), camphene (0.5%), *l*-β-pinene (3.5%), sabinene (0.5%), Δ^3-carene (0.3%), β-myrcene (1.2%), α-phellandrene (0.1%), *l*-limonene (0.7%), *l*-β-phellandrene (0.2%), γ-terpinene (0.3%), p-cymene (0.2%), terpinolene (0.1%), methyl n-nonyl ketone (2.4%), d-thujone (0.3%), *l*-camphor (0.2%), d-terpinen-4-ol (0.3%), *l*-linalool (0.7%), *l*-borneol (0.3%), *l*-terpineol (0.4%), nerol (0.2%), geraniol (0.25%), d-cedrol (12%), elemol (0.3%), eudesmol (0.35%), and sesquiterpene hydrocarbons (5.6%): *l*-α-cedrene, *l*-β-elemene, *l*-β-cedrene, d-γ-cadinene, d-ar-curcumene, *l*-calamenene, and d-calacorene.

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