CAPILLARY GAS CHROMATOGRAPHIC ANALYSIS OF THE MONOTERPENOIC FRACTION OF ESTONIAN CONIFER NEEDLE OIL

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Abstract. The qualitative and quantitative variation of the monoterpenoic compounds in Estonian conifer needle oils was studied using capillary gas chromatography. The Scots pine trees in Estonia belong to a high 3-carene chemotype with the content of 3-carene in the needle oil amounting to about 20–60% of the total monoterpene fraction. The Norway spruce needle oil contains camphene, limonene, and 1,8-cineole as the main components.

Key words: capillary gas chromatography, monoterpenes, conifer needle oil.

Scots pine (*Pinus sylvestris* L.) appears to be one of the most complicated species of the genus *Pinus*. Namely, it has been found that two neighbouring trees of the same biological subspecies *Pinus sylvestris* L. subsp. *sylvestris* L. may produce a completely different monoterpenoic (MT) composition of their oleoresins and essential needle oils. The occurrence of trees with a high α-pinene content (more than 70%), trees with a high 3-carene content (30–50%), and trees containing relatively much β-pinene (more than 10%) has been established [1–3]. These variations have been found to be due to the genetic differences between individual trees as their monoterpene biosynthesis proceeds under strong genetic control [4–7].

Essential spruce oils are characterized by a relatively high amount of oxygenated monoterpenes and a combination of α-pinene, β-pinene, β-phellandrene, camphene, myrcene, and limonene as the main monoterpenes [8–10]. Little information is available on the needle oil composition of Norway spruce (*Picea abies* K.) widely growing in Estonia.

We investigated the qualitative and quantitative variation of the monoterpenoic compounds in Estonian conifer needle oils using high resolution capillary gas chromatography (GC).

EXPERIMENTAL

Since the Estonian conifer needle oil has not been studied earlier by gas chromatography, we decided to establish first how big the differences in essential oil compositions for individual trees could be. We gathered needles (20–50 g) from pines and spruces growing in different places in Estonia (Valga, Ida-Virumaa, Jõgeva, Tallinn).

The essential oils were isolated using a combined steam distillation and extraction (SDE) Marcusson microapparatus. The oil fraction in n-hexane (1–5 µl) was analysed on two fused silica capillary columns (50 m × 0.25 mm) with bonded stationary phases (OV-101, PEG 20M). The chromatographic system comprised a gas chromatograph Chrom 5, flame ionization detector, and data system (Hewlett-Packard Model 3390A integrator). Helium with a flow rate of about 0.3 ml/min for OV-101 and 1.5 ml/min for PEG 20M was used as the carrier gas. The column temperature was programmed from 50 to 220°C (OV-101) and from 70 to 220°C (PEG 20M) at 2°/min. The individual MT compounds were identified according to their retention indices on two columns in comparison with authentic data either determined in our laboratory or obtained from literature [11, 12]. The results obtained were checked by chromato-mass spectrometry. The quantitative composition of individual compounds in the MT fraction was calculated using their peak areas. The mass-spectrometric analysis was carried out on a Hitachi M-80B gas chromatograph double focussing mass-spectrometer using RSL 300 capillary column (30 m × 0.32 mm). The temperature was programmed from 60 to 120°C at 5°/min, and then to 290°C at 10°/min.

RESULTS AND DISCUSSION

Errors arising from SDE were studied by analysing the standard mixtures under possibly the same conditions as needles. The coefficient of variation determined on the basis of GC analysis of standard mixtures before and after SDE did not exceed 10% except for some minor compounds (< 1%). The coefficient of variation for the repeated GC analyses of single oil samples was below 5%.

n-Tetradecane was used as the internal standard for the determination of oil yield. The amount of essential oils found in the needles was about 1% of the dry weight for pine and up to 0.5% for spruce. The typical chromatograms for pine and spruce needle oils are presented in the Figure.

The pine needle oils contained besides monoterpenes 5–40% sesquiterpenes (molecular mass mainly 204). The spruce needle oils contained in addition to monoterpenes 20–50% terpenoic alcohols and esters and only a small percentage of sesquiterpenes.

In this work only the composition of the monoterpene fraction of conifer needle oils is discussed. The MT composition of five characteristic samples of pine and spruce needle oils is presented in the Table. The total

amount of MT for spruce was found to be somewhat smaller (40–60%) than for pine (60–90%). Eighteen compounds were identified in the MT fraction of conifer needle oils, with α-pinene and 3-carene presented in the greatest amounts in pine needle oil and camphene, limonene, and 1,8-cineole in spruce needle oil.
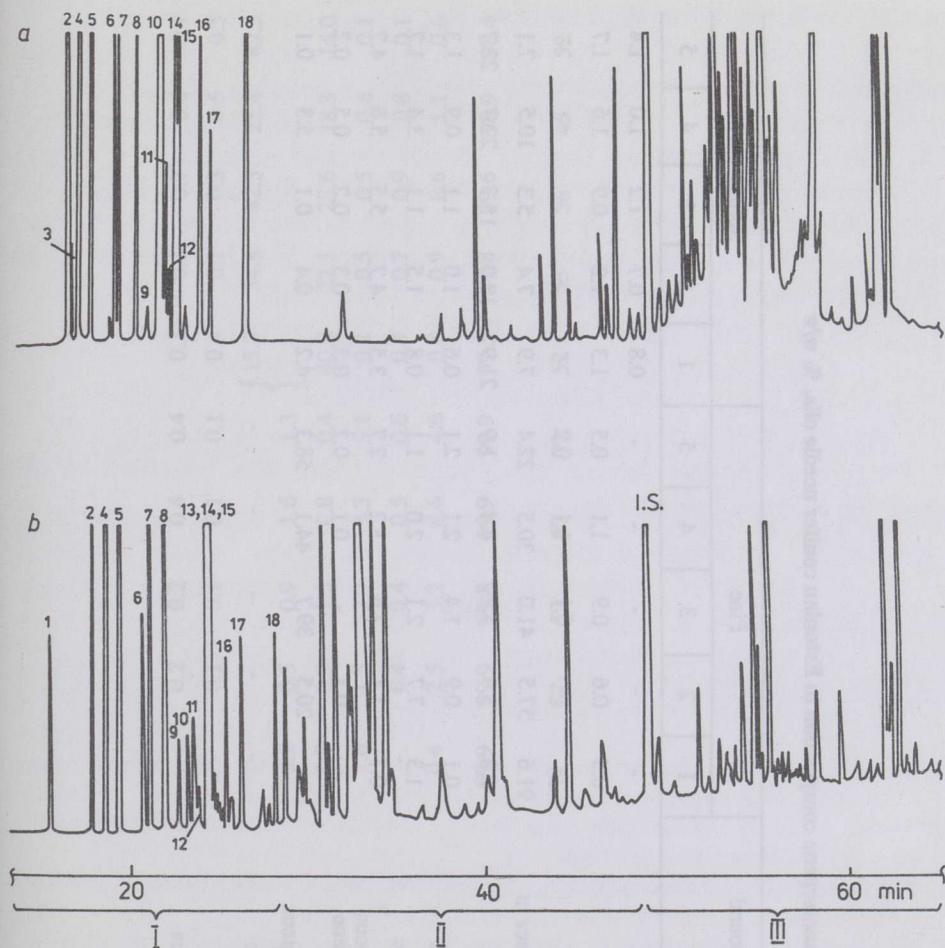
Chromatograms of pine needle oil of sample No. 3 (a) and spruce needle oil of sample No. 2 (b) obtained on the OV-101 column. Peak numbers refer to the compounds listed in the Table. IS = internal standard; I, monoterpenes; II, monoterpenoic alcohols and esters; III, sesquiterpenes.

Pine No. 1 is an α-pinene-rich tree. It is a planted tree growing at Õismäe and the origin of its seeds is not known. The other pine trees represent the high 3-carene chemotype where the content of 3-carene makes up about 20–60% of the total MT fraction.

The only naturally growing spruce in Estonia is Norway spruce. As all our samples were gathered from the forest, they must belong to Norway spruces. However, the composition of all our samples differed notably from all those discussed in the literature [8–12].

Our spruce samples contained 15–40% of limonene and 15–40% of a component with a retention index equal to that of β-phellandrene on both columns (OV-101 and PEG 20M). It is generally known [13] that all

Monoterpene composition in Estonian conifer needle oils, % w/w

Peak number	Compound	Pine					Spruce				
		1	2	3	4	5	1	2	3	4	5
1	Santene	-	-	-	-	-	0.8	0.7	1.2	1.0	1.4
2	Tricyclene	0.5	0.6	0.9	1.1	0.5	1.3	1.2	0.9	1.6	1.7
3	α-Thujene	-	-	0.1	1.1	0.2	-	-	-	-	-
4	α-Pinene	91.6	57.5	41.0	30.5	22.4	7.9	7.4	5.3	10.5	7.1
5	Camphene	1.8	3.4	3.2	4.4	1.9	21.9	18.0	15.3	22.7	22.7
6	Sabinene	0.1	0.9	1.4	2.1	2.1	0.6	1.0	1.1	0.9	1.3
7	β-Pinene	1.3	7.7	2.1	2.0	1.1	0.8	1.5	1.1	3.4	1.7
8	Myrcene	0.7	3.1	2.4	3.2	2.7	3.5	4.7	5.5	5.6	4.7
9	α-Phellandrene	-	0.1	-	0.1	0.2	0.3	0.3	0.2	0.5	0.2
10	3-Carene	-	20.5	39.7	44.1	58.3	4.2	0.4	0.1	5.3	0.1

component No. 13 in the spruce needle oil could be β-phellandrene. Yet the mass spectrometer data showed clearly that this component was 1,8-cineole. We have not found any reference showing 1,8-cineole in such a large amount as the spruce needle oil.

According to their monoterpene composition the needles of the Scots pine trees in Estonia belong to a high 3-carene group. The Norway spruce needle oil contains camphene, α-pinene and 1,8-cineole as the main constituents.

11 α-Terpinene	-	-	0.2	0.2	0.4	0.4	0.3	0.5	0.7	0.6	0.7
12 p-Cumene	-	-	0.1	0.1	0.1	0.1	0.4	0.1	0.3	0.6	0.5
13 1,8-Cineole	-	-	-	-	-	15.3	34.8	42.3	42.3	25.4	42.5
14 β-Phellandrene	0.2	0.2	0.5	0.9	1.6	1.3	-	-	-	-	-
15 Limonene	1.7	1.7	0.9	1.5	0.8	3.1	40.8	27.1	23.6	18.3	14.0
16 trans-Ocimene	1.6	1.6	1.5	2.7	3.3	0.5	0.2	0.5	0.5	0.6	0.1
17 γ-Terpinene	0.4	0.4	0.4	0.4	0.5	0.6	0.4	0.7	0.9	0.8	0.1
18 Terpinolene	0.4	2.5	4.6	4.6	4.6	0.8	0.8	0.9	0.6	1.1	0.6
Total	99.9	99.9	99.8	99.8	99.9	99.9	99.5	99.8	99.6	98.9	98.4
Monoterpenes in needle oil	71	87	57	57	67	72	52	53	56	45	52

- not detected

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conifer trees irrespective of their species produce the same MT compounds (in different quantities, of course). So we supposed that component No. 13 in the spruce needle oil could be β-phellandrene. Yet the mass-spectrometric analysis showed clearly that this component was 1,8-cineole. We have not found any reference showing 1,8-cineole in such a large amount in the spruce needle oil.

CONCLUSIONS

According to their monoterpene composition the needle oils of the Scots pine trees in Estonia belong to a high 3-carene group. The Norway spruce needle oil contains camphene, limonene, and 1,8-cineole as the main components.

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EESTI MÄNNI JA KUUSE OKKAÕLI MONOTERPEENSE OSA KAPILLAARGAASIKROMATOGRAAFILINE ANALÜÜS

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Kapillaargaasikromatograafia meetodil on uuritud Eesti männi ja kuuse okkaõlide monoterpeense osa kvalitatiivset ja kvantitatiivset koostist. Kuuse okkaõli monoterpeenses osas identifitseeriti põhikomponentidena limoneen, kamfeen ja massispektri järgi 1,8-tsineool, mida varem pole nii suurel hulgal kuuse okastes leitud. Uuritud männid kuulusid põhiliselt kareenirikaste mändide rühma.

КАПИЛЛЯРНАЯ ГАЗОВАЯ ХРОМАТОГРАФИЯ МОНОТЕРПЕНОВОЙ ФРАКЦИИ ЭФИРНЫХ МАСЕЛ ДЕРЕВЬЕВ ХВОЙНЫХ ПОРОД ЭСТОНИИ

Анне ОРАВ, Тийу КАЙЛАС, Милана ЛИЙВ, Рийна ААВ

Изучены качественные и количественные изменения монотерпеновой части эфирных масел эстонских хвойных пород при помощи капиллярной газовой хроматографии. Установлено, что по составу монотерпенов сосна обыкновенная лесная может быть отнесена к группе каренистых. Основными компонентами монотерпеновой части эфирных масел хвои ели обыкновенной являются камфен, лимонен и 1,8-цинеол.