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NMR ANALYSIS OF THE COMPOSITION
OF ESSENTIAL OIL OF FUR DURING ITS EVAPORATION

Comparative analysis of the composition of essential oil of fir during its evaporation in air for 12 weeks by method of NMR ^{1}H and ^{13}C was carried out. 12 major essential oil components were identified and quantified. It was found that the compositional change of essential oil is reflected experimentally in the quantitative content of components, and caused mainly by evaporation of highly volatile compounds. The oxidation and polymerization processes in new products approve themselves slightly.

Introduction. Essential oil of spruce stimulates the immune system, it relaxes and helps to cope with nervousness. It can provide faster recovering from illness and due to high content of bornyl acetate spruce essential oil has significant antimicrobic action, especially against staphylococcus.

During storage, there may be a change in the properties of essential oil caused by light and oxygen as its components readily undergo oxidation, isomerization and polymerization. Furthermore, because of the different volatility of the components their quantitative content in the mixture must be changed.

At present, chromatographic methods are mainly used for controlling the composition and quality of oils [1,2]. Despite their versatility, to get reliable results for the analysis of complex mixtures one must use columns with various fillers and to have the individual components of the mixture being analyzed, thereby increasing analysis time.

In this regard high-resolution NMR spectroscopy has significant advantages. Modern spectrometers have high sensitivity and allow to analyze complex structures on different cores in a relatively short period of time, giving reliable results [3].

The aim of this work is a comparative analysis of the composition of essential oil of spruce in the process of evaporation by NMR method.

Main part. Samples of needles were selected in autumn and winter months from spruce growing in the district of Minsk. During this period the yield of essential oil reaches the maximum value, and its structure is stabilized [4].

Table 1

Chemical shifts [1]H (δ, ppm) of the components of essential oils of common spruce needles.

Compound	Atomic number of carbon											
	1	2	3	4	5	6	7	8	9	10	11	12
I	–	7.13	7.15	–	7.15	7.13	2.34	2.89	1.26	1.26	–	–
II	5.27; 5.08	6.40	–	2.25	2.22	5.18	–	1.64	1.73	5.04; 5.03	–	–
III	–	6.16	5.76	2.08	1.78; 1.41	2.46; 2.31	4.78; 4.76	1.67	0.93	0.94	–	–
IV	–	5.42	2.08; 1.91	2.11	1.81; 1.50	2.05; 1.97	1.67	–	4.73	1.75	–	–
V	0.64	2.36; 1.97	–	5.26	2.19; 1.81	0.74	–	1.62	1.05	0.79	–	–
VI	1.96	–	5.20	2.26; 2.19	2.10	–	2.37; 1.19	1.69	1.30	0.87	–	–
VII	–	4.82	2.30; 0.91	1.62	1.69; 1.19	1.89; 1.25	–	0.85	0.82	0.78	–	2.01
VIII	2.70	–	–	1.93	1.73; 1.42	1.67; 1.27	1.72; 1.23	1.09	1.06	4.75; 4.52	–	–
IX	2.48	–	2.55; 2.27	1.87; 1.84	2.00	–	2.34; 1.45	4.65; 4.59	1.27	0.75	–	–
X	–	3.98	2.25; 0.93	1.61	1.71; 1.23	1.88; 1.23	–	0.85	0.84	0.83	–	–
XI	–	–	2.29; 1.78	2.03	1.89; 1.29	1.62; 1.34	–	0.85	0.90	0.77	–	–
XII	–	1.58; 1.41	1.93; 1.41	1.32	1.93; 1.41	1.58; 1.41	0.93	–	1.15	1.15	–	–

Table 2

Chemical shifts ^{13}C (δ,ppm) of the components of essential oils of common spruce needles

Compound	Atomic number of carbon											
	1	2	3	4	5	6	7	8	9	10	11	12
I	135.8	129.7	126.9	146.5	126.9	129.7	21.6	34.4	24.8	24.8	–	–
II	113.7	139.7	146.8	32.1	27.4	124.8	132.4	26.4	18.4	116.3	–	–
III	134.8	121.4	30.2	128.2	27.3	32.1	20.8	122.3	24.1	20.3	–	–
IV	134.3	121.3	31.5	41.8	28.6	31.3	24.1	150.8	109.1	21.5	–	–
V	17.4	21.5	132.0	120.1	25.5	19.2	17.4	24.3	29.0	13.9	–	–
VI	47.7	145.2	116.7	32.2	41.4	38.7	32.0	23.7	27.1	21.5	–	–
VII	49.2	80.4	37.3	45.4	28.6	27.6	48.3	20.3	19.4	14.0	171.9	21.8
VIII	47.6	166.9	42.5	48.8	24.5	29.6	38.1	30.1	26.6	99.8	–	–
IX	52.5	152.7	24.3	24.3	41.1	41.3	27.6	106.7	26.8	22.5	–	–
X	50.1	77.9	39.6	45.7	28.9	26.6	48.6	20.8	19.3	14.0	–	–
XI	58.2	220.2	43.8	43.6	27.6	30.4	47.2	9.8	20.3	19.7	–	–
XII	74.1	32.0	23.3	33.4	23.3	32.0	28.1	70.2	29.4	29.4	–	–

The selected needles were separated from stipes and reduce to fine particles of ~ 5mm, and by hydro-distillation method essential oil was distilled, which was then dried using calcium chloride. The resulting essential oil in an amount of 15 g was poured into a beaker of 100 ml and a diameter of 40 mm.

Curing temperature for open glass is $(18 \pm 1)°$C for 12 weeks. After certain intervals, 0.03 g was sampled and dissolved in 0.5 ml of deuterochloroform for analysis by NMR spectroscopy. Before carrying out the analysis, the glass with essential oil was weighed.

NMR spectra were recorded in quantitative methods on spectrometer AVANCE-500 (Bruker) with an operating frequency of 500 and 126 MHz for nuclei ^{1}H and ^{13}C, respectively. Recording was performed at a temperature of 293 K, as an internal standard in the spectra of ^{1}H signal CHCl$_3$ (δ = 7,27 ppm) was used, ^{13}C – solvent signal (δ = 77,7 ppm).

For assignment of signals in the spectra correlation spectroscopy techniques COSY-45, NOESY, HSQC and HMBC were used.

All the experimental data were obtained and processed using the software package XWIN-NMR 3.5.

For identification and quantitative determination of the components of essential oils spectra of various terpene compounds were recorded, 12 of which were present in appreciable amounts in the samples and were analyzed by us.

Since the studied compounds are multispin systems and have rather complex NMR spectra, complete assignment of signals in the spectra was done. Tables 1 and 2 give the chemical shifts of ^{1}H and ^{13}C NMR newly identified terpenes. Literature data on the chemical shifts of ^{1}H and ^{13}C NMR for some of the compounds are in good agreement with the results obtained.

Visual observation of evaporating essential oil showed that a polymer film appeared on its surface after 4 weeks.

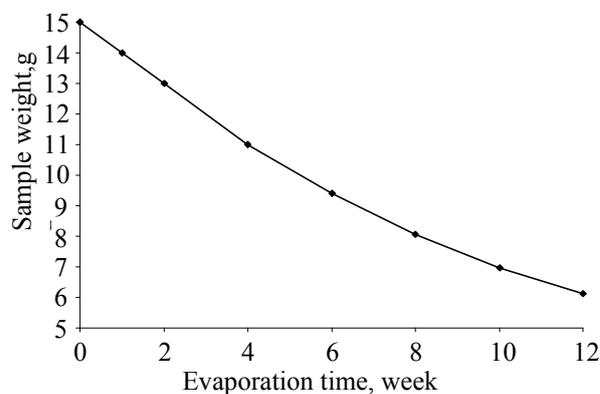Fig. 1 shows the dependence of the change in the sample weight from evaporation time.

Fig. 1. The dependence
of the sample weight
from evaporation time

The figure shows that this dependence is linear for the first four weeks, then the evaporation rate decreases, which is apparently due to the removal of more volatile components and the appearance of the plastic film preventing from evaporation.

Changing of the composition is established by means of ^{1}H NMR spectroscopy. Figure 2 shows the spectra: *a* – source of essential oil, *b* – after 12 weeks of evaporation.
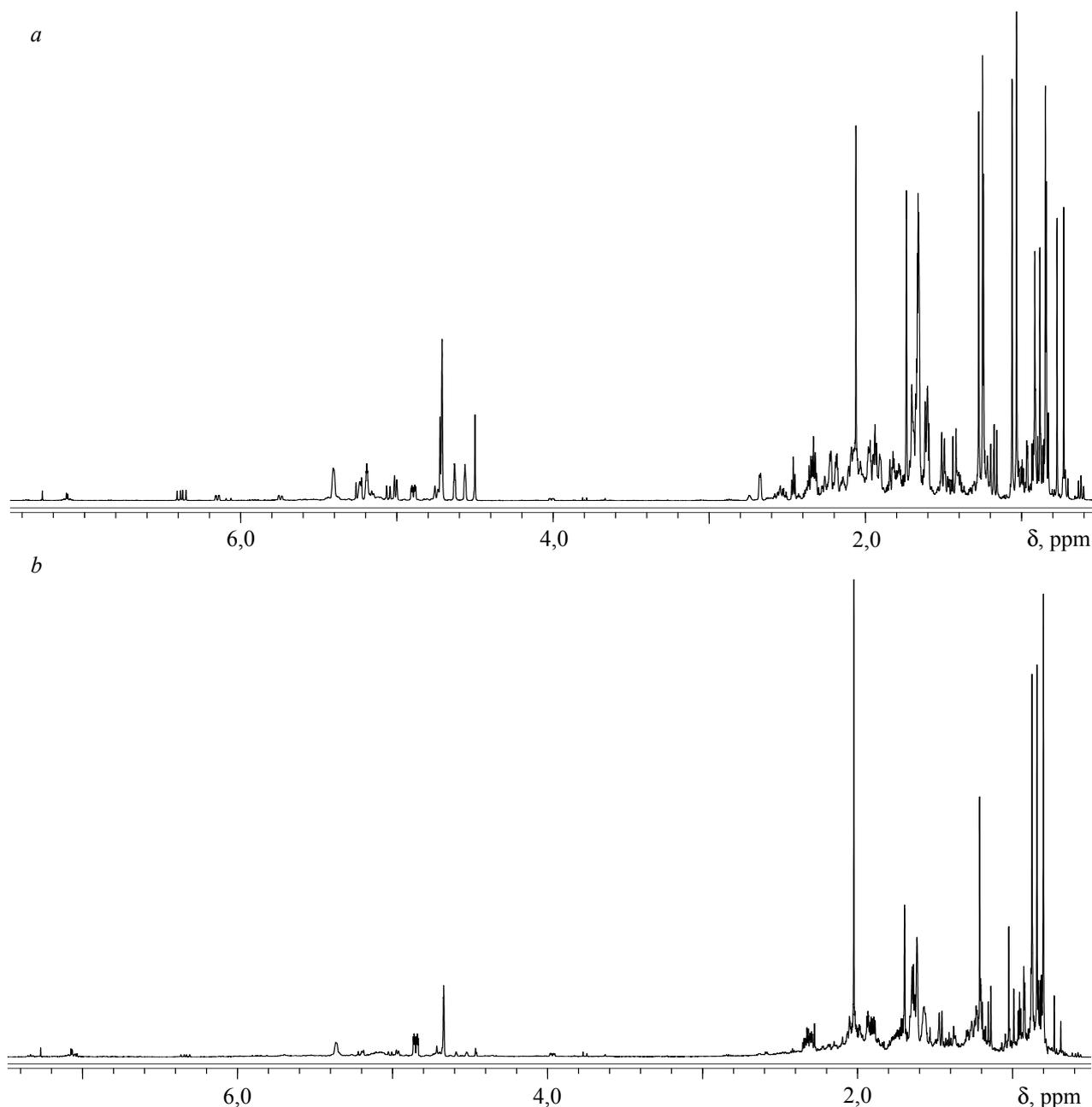
Fig. 2. ^{1}H NMR spectra of solutions in CDCl$_3$ spruce essential oils:
a – the original sample; *b* – the sample after evaporation for 12 weeks

The spectra are notably different and mainly decreasing intensity of series of lines in the course of time. New lines were not found out, but in the aromatic protons field the intensity of the lines of unidentified components increased.

Similar results are obtained by analysis of the spectra ^{13}C (Fig. 3): *a* – initial essential oil, *b* – after 12 weeks of evaporation. However, due to the large spectral range, one can be claimed that along with evaporation oxidation occurs, as new lines appear $\delta = 62.62$ and 73.30 ppm corresponding to oxygen compounds.

Furthermore, the wide base below the lines located in the range of 18–35 ppm, may refer to the methine and methylene carbon atoms of polymeric compounds.

However, these changes are not prevailing compared with a simple decrease in the content of some components.

The study of the proton and carbon spectra of essential oil of spruce needles showed that its composition is dominated by compounds whose structural formulas are shown in Fig. 4. The above numbering of carbon atoms of these components corresponds to the numbering in tables 1 and 2 respectively. These compounds are paracymol (I), myrcene (II), β-phellandrene (III), limonene (IV), Δ^3-carene (V), α (VI), bornyl acetate (VII), cam-

phene (VIII), β-pinene (IX), borneol (X), camphor (XI) and eucalyptole (XII).

Table. 3 shows the structure of essential oils spruce needles, depending on the terms of evaporation and the boiling point of its components.

Analysis of the table shows that the part of the identified components (94.6–89.2%) gradually de-creases, as the evaporation of volatile oil apparently due to increasing the proportion of seksviterpenes which have not been analyzed, as well as oxidized and polymeric products.

However, even in the last sample (40,9% of the original weight) identified starting compounds prevail.
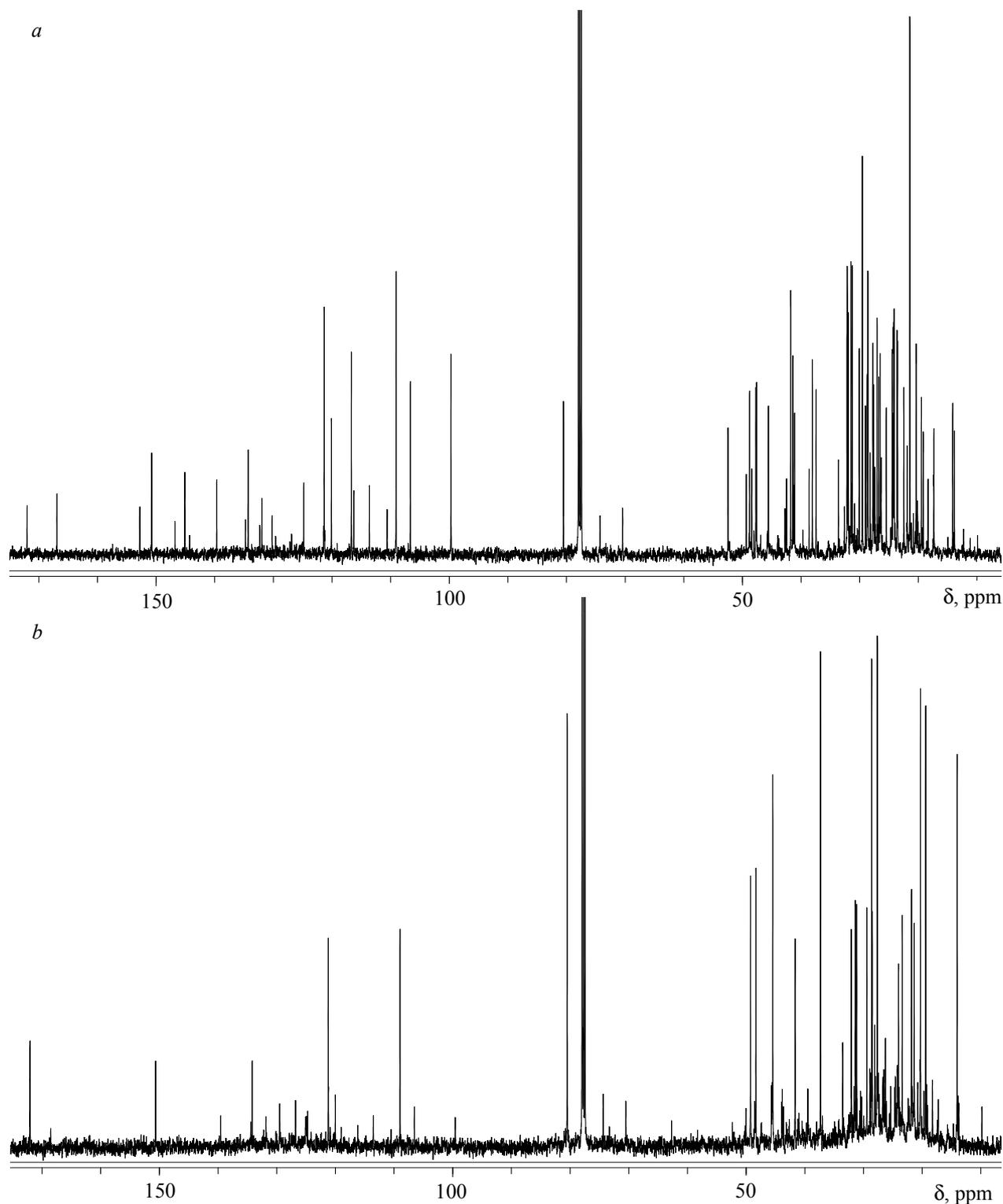

Fig. 3. ^{13}C NMR spectra of solutions in CDCl$_3$ spruce essential oils:
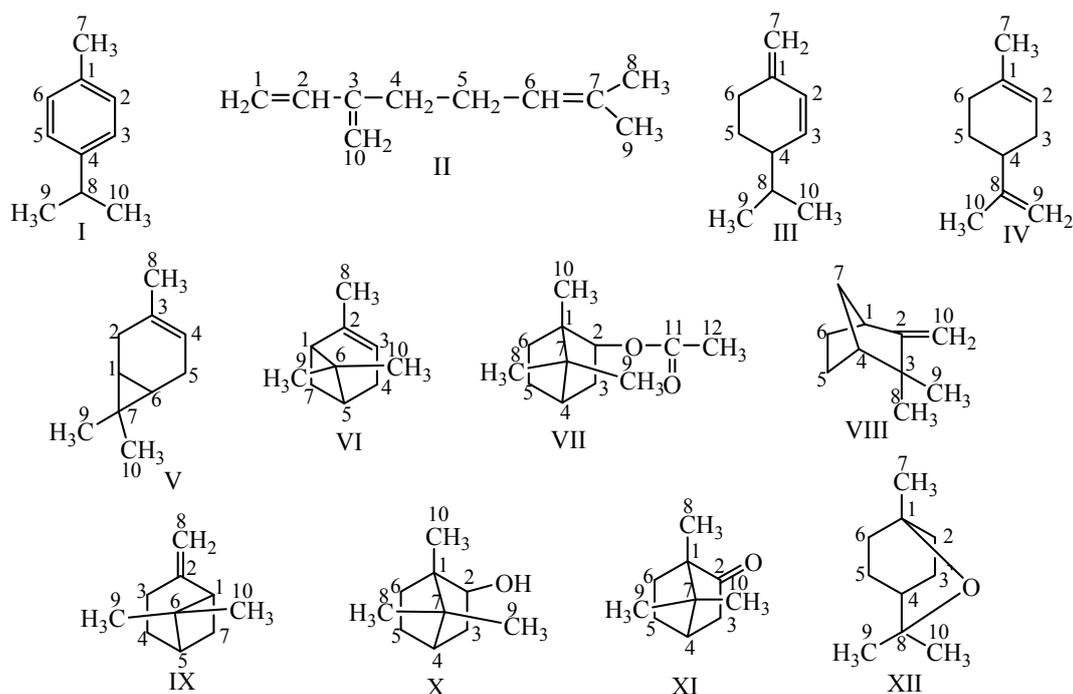*a* – the original sample; *b* – the sample after evaporation for 12 weeks

Fig. 4. The structural formulas of the main components of spruce needles essential oil

Table 3

The composition of the essential oil of spruce depending on the evaporation time (e.g., a week)

Compound	$T_{boiling}$, °C	Content, molar %							
		starting	1w	2w	4w	6w	8w	10w	12w
I	177	0.7	0.8	1.0	1.3	1.5	1.6	1.8	1.9
II	167	4.6	4.5	4.3	4.1	3.9	3.7	3.5	3.4
III	172	2.7	2.6	2.5	2.3	2.2	2.1	2.0	1.9
IV	176	17.7	18.5	19.3	20.2	20.5	20.4	19.4	16.2
V	172	8.8	8.4	7.9	7.2	6.6	6.1	5.5	5.2
VI	156	16.4	14.8	13.2	10.3	8.2	6.3	4.2	3.0
VII	225	10.7	11.6	13.0	15.9	18.2	21.6	28.1	34.6
VIII	159	13.1	12.4	11.6	9.7	8.1	6.0	3.2	1.5
IX	164	11.2	11.0	10.7	9.8	8.6	7.0	5.0	3.5
X	212	1.3	1.5	1.7	2.1	2.4	3.2	4.7	5.9
XI	204	1.2	1.4	1.5	1.8	2.1	2.3	2.5	2.6
XII	176	6.2	7.0	7.6	8.4	8.8	9.3	9.5	9.5

Dynamics of changes in the content of components correlates well with the boiling point of compounds. So the content of the most low-boiling compounds – α-pinene and camphene – most strongly reduced in the mixture, but the high-boiling compounds – bornyl acetate and borneol – most intensively increases.

Conclusion. Thus, evaporation of essential oil spruce needles at room temperature in an open vessel is practically a physical process with an insignificant share of oxidation and polymerization processes.

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