

CHEMOMETRIC ANALYSIS OF CONIFER NEEDLE OILS

Anne ORAV, Tiiu KAILAS, and Mihkel KOEL

Institute of Chemistry, Akadeemia tee 15, EE-0026 Tallinn, Estonia

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Abstract. Principal component analysis was applied on the chromatographic profiles of 30 major components of needle oils of 13 spruce and 12 pine trees from different environments. The analysis revealed no dependence on polluted environment. The correlation coefficients between pairs of monoterpenes gave some information about the formation of individual terpenoid compounds and their mutual dependence or independence in the synthesis pathway.

Key words: capillary gas chromatography, conifer needle oils, terpenoid composition, chemometric analysis.

INTRODUCTION

In our previous works we studied the terpenoid composition of Estonian conifer needle oils [1, 2]. The results of those investigations showed high variation of terpenoid concentrations for individual trees. There is no satisfactory explanation for such big differences and hypotheses that these variations may arise from the genetic and/or environmental effects have been put forward. Statistical analysis of variations in the terpenoid composition of essential oils seems to be the best tool for studying the causes of such variations [3–10]. Juvonen [4] and Hiltunen [5, 10] investigated the biosynthetic pathways involved in the formation of monoterpenes in Scots pine needles by examining interrelationships of monoterpenes. Yazdani and coworkers [6, 7] presented the genetic expression for monoterpenes in the clones of *Pinus sylvestris* using different statistical methods (principal component analysis, cluster analysis, genotype correlation, etc.). Regimbal & Collin [8] studied the essential oil of Balsam fir by

principal component factor analysis. Gas chromatograms of oils extracted from Eucalyptus species were compared by Dunlop et al. [9] using principal component analysis, linear discriminant analysis, and the Tanimoto metric.

In the present work some of the above-mentioned statistical methods were applied to study the genetic and environmental effects on the variation of the terpenoid composition in Estonian conifer needle oils.

RESULTS AND DISCUSSION

The needles were collected from 12 pine and 13 spruce trees growing under different environmental conditions (in a forest, close to a city, road, and a chemical factory) in Estonia. Essential oils were isolated using a simultaneous steam distillation and extraction micro-apparatus. The extracts obtained were analysed by gas chromatography on two fused silica capillary columns (OV-101, PEG 20M) as described in [1, 2]. We used the qualitative and quantitative results of those studies as database for the present work taking into consideration that according to the composition of their monoterpenes the needle oils of Scots pine trees in Estonia belong to a high carene chemotype. Big differences in terpenoid concentrations for individual trees among a population in Estonia (Fig. 1) were found for both spruce and pine needle oils. In spruce needle oils large quantitative variations were characteristic of all main terpenoid components (camphene, limonene, 1,8-cineole, camphor, bornyl acetate, camphene hydrate). In pine needle oils the ranges of quantitative variance of α -pinene and 3-carene, the components with the highest concentrations, were larger than for other oil components. To examine the causes of such large quantitative variance between individual trees we used principal component analysis and component pairs correlation.

The results of principal component analysis for 32 terpenoid components in the needle oils of 12 pine trees and for 27 components in the needle oils of 13 spruce trees are presented in Fig. 2. These plots show that the differences in the terpenoid patterns obtained from different environmental conditions were statistically insignificant. Obviously the environmental effect on the terpene content is very small or the environmental pollution in Estonia is not high enough to affect terpenoid concentrations. This fits very well with Hiltunen [5], who concluded that the greatest part of the total variation in the terpenoid concentrations is due to the genetic differences between individual trees.

The correlation coefficients between pairs of monoterpenes in spruce and pine needle oils are presented in Tables 1 and 2. Strong positive correlations were found between 3-carene and α - and γ -terpinene, terpinolene, *trans*-ocimene, and sabinene. All these compounds were present in much greater amounts in the 3-carene

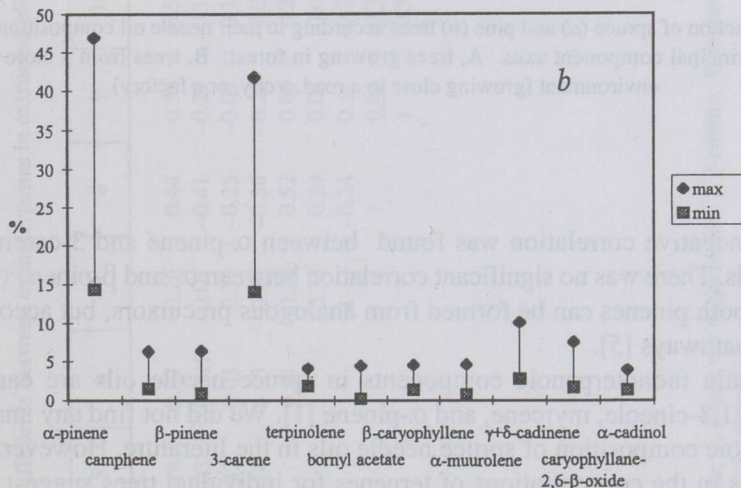
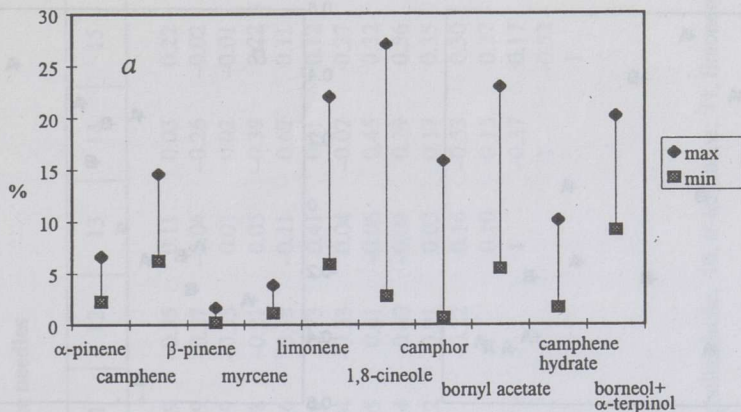


Fig. 1. Variation of the relative content of major components in spruce (a) and pine (b) needle oil.

chemotype than in the pine one [5, 10]. This result is in agreement with Juvonen [4], who proposed that these compounds form via the same biosynthetic route.

Tricyclene and camphene had correlation coefficients higher than 0.9 as reported by Regimbal & Collin [8] for Balsam fir oil, but there was no correlation between α -pinene and camphene or tricyclene in Estonian pine needle oils. Also α - and β -phellandrene and limonene were shown to be biosynthetically linked.

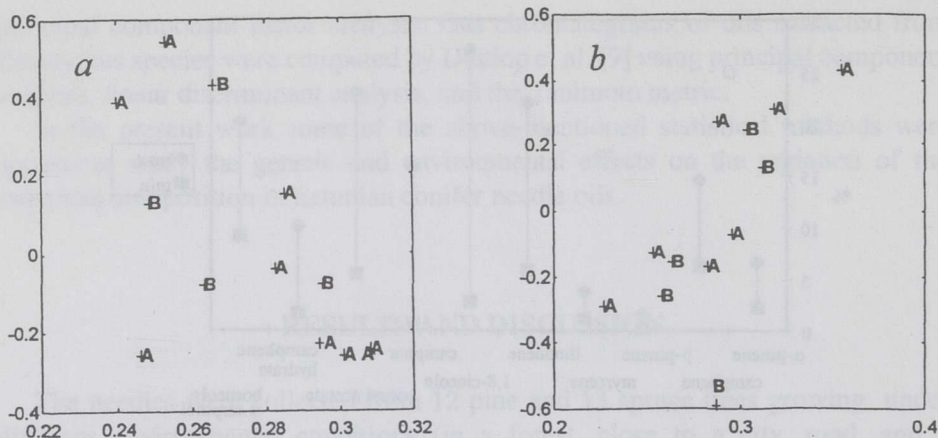


Fig. 2. Projection of spruce (a) and pine (b) trees according to their needle oil composition onto the first two principal component axes. A, trees growing in forest; B, trees from a more polluted environment (growing close to a road, a city, or a factory).

A strong negative correlation was found between α -pinene and 3-carene group compounds. There was no significant correlation between α - and β -pinene ($r = 0.6$), although both pinenes can be formed from analogous precursors, but according to different pathways [5].

The main monoterpene components in spruce needle oils are camphene, limonene, 1,8-cineole, myrcene, and α -pinene [1]. We did not find any analysis of the terpenoid composition of spruce needle oils in the literature. However, the big differences in the concentrations of terpenes for individual trees suggest that the processes that control the formation of terpenes in spruce needles could be analogous to those of pine trees.

The results of correlation analysis of monoterpene pairs of spruce needle oils revealed a strong positive correlation between α -pinene, tricyclene, and camphene (not found in pine needles oils). Also α - and γ -terpinene as well as myrcene and α -phellandrene were shown to be linked. There were no components in spruce needles with strong negative correlation.

The correlation coefficients between monoterpene–sesquiterpene pairs and between sesquiterpene pairs were also determined. A strong positive correlation was observed only between β -caryophyllene and α -humulene ($r > 0.9$). There was no significant correlation between other pairs of oil components.

Correlation coefficients between monoterpenes in extracted oils of spruce needles

X	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1	0.24	0.24	0.24	0.18	0.37	-0.25	0.44	0.31	0.51	-0.19	-0.35	0.11	0.03	0.22
2		1	0.86	0.92	-0.03	0.001	0.01	-0.41	-0.29	-0.17	-0.19	-0.27	-0.04	-0.26	-0.02
3			1	0.79	0.32	-0.03	0.33	-0.25	-0.09	-0.17	-0.29	-0.35	0.01	0.02	-0.01
4				1	-0.14	0.03	0.13	-0.36	-0.25	-0.06	0.08	-0.21	0.05	-0.39	0.22
5					1	0.24	0.28	0.52	0.65	0.25	-0.66	0.18	-0.11	0.67	0.11
6						1	-0.33	0.34	0.05	0.66	-0.29	0.73	0.41	0.11	0.12
7							1	-0.24	0.11	-0.25	-0.04	-0.33	0.04	-0.02	0.27
8								1	0.83	0.75	-0.05	0.61	-0.06	0.45	0.32
9									1	0.51	-0.14	0.40	-0.09	0.29	0.56
10										1	-0.12	0.91	0.03	0.17	0.35
11											1	-0.22	0.16	-0.53	0.30
12												1	0.10	0.15	0.27
13													1	-0.37	0.17
14														1	-0.52
15															1

1, santene; 2, tricyclene; 3, α -pinene; 4, camphene; 5, β -pinene; 6, sabinene; 7, 3-carene; 8, myrcene; 9, α -phellandrene; 10, α -terpinene; 11, limonene; 12, γ -terpinene; 13, *trans*-ocimene; 14, *p*-cymene; 15, terpinolene.

Correlation coefficients between monoterpenes in extracted oils of pine needles

X	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	-0.29	<u>0.97</u>	-0.05	-0.03	0.001	0.45	-0.14	-0.09	-0.28	-0.25	0.09	-0.32	-0.15
2		1	-0.21	0.58	<u>-0.84</u>	<u>-0.79</u>	-0.06	0.14	-0.52	0.47	0.16	-0.59	-0.54	-0.66
3			1	0.07	-0.09	<u>-0.07</u>	0.47	-0.13	-0.09	-0.22	-0.24	0.11	-0.38	-0.14
4				1	-0.49	-0.45	0.49	-0.01	-0.01	0.20	0.02	-0.13	-0.38	-0.30
5					1	<u>0.95</u>	0.09	-0.18	0.67	-0.55	-0.14	<u>0.71</u>	<u>0.81</u>	<u>0.79</u>
6						1	0.03	-0.31	<u>0.71</u>	-0.66	-0.32	<u>0.76</u>	<u>0.84</u>	<u>0.79</u>
7							1	0.24	0.30	-0.02	0.22	0.26	-0.19	0.11
8								1	0.01	0.74	0.90	-0.06	-0.48	0.19
9									1	-0.45	-0.15	0.95	0.57	0.87
10										1	0.86	-0.54	-0.57	-0.24
11											1	-0.25	-0.36	0.09
12												1	0.54	<u>0.88</u>
13													1	0.61
14														1

1, tricyclene; 2, α -pinene; 3, camphene; 4, β -pinene; 5, sabinene; 6, 3-carene; 7, myrcene; 8, α -phellandrene; 9, α -terpinene; 10, limonene; 11, β -phellandrene; 12, γ -terpinene; 13, *trans*-ocimene; 14, terpinolene.

CONCLUSIONS

The factor analysis of chromatographic data of the major components of needle oils of Estonian pine and spruce trees showed that the differences in terpenoid concentrations of individual trees were not markedly correlated to the environmental pollution. Correlation analysis of component pairs showed strong positive correlation and a genetic link between 3-carene, α - and γ -terpinene, terpinolene, and sabinene; between tricyclene and camphene; between α - and β -phellandrene and limonene for pine needle oils. Between sesquiterpenoid pairs the biggest correlation coefficient was found between α -humulene and β -caryophyllene. For spruce needle oils a strong positive correlation was observed between α -pinene, tricyclene, and camphene.

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REFERENCES

1. Orav, A., Kailas, T., Liiv, M. & Aav, R. Capillary gas chromatographic analysis of the monoterpenoid fraction of Estonian conifer needle oil. *Proc. Estonian Acad. Sci. Chem.*, 1995, **44**, 2/3, 149–155.
2. Orav, A., Kailas, T. & Liiv, M. Analysis of terpenoid composition of conifer needle oils by simultaneous distillation–extraction, gas chromatography and gas chromatography–mass spectrometry. *Chromatographia*, 1996, **43**, 3/4, 215–219.
3. Rudloff, E. Chemosystematic studies in the genus *Picea* (Pinaceae). *Can. J. Bot.*, 1967, **45**, 6, 891–901.
4. Juvonen, S. Über die Terpenbiosynthese beeinflussenden Faktoren in *Pinus sylvestris* L. *Acta Bot. Fenn. Societas pro fauna et flora Fennica*, Helsinki, 1966, **71**, 1–92.
5. Hiltunen, R. On variation, inheritance and chemical inter-relationships of monoterpenes in Scots pine (*Pinus sylvestris* L.). *Ann. Acad. Sci. Fenn., Ser. A IV, Biol.*, 1976, **208**, 1–54.
6. Yazdani, R. & Nilsson, J.-E. Cortical monoterpene variation in natural populations of *Pinus sylvestris* in Sweden. *Scand. J. For. Res.*, 1986, **1**, 85–93.
7. Baradat, Ph. & Yazdani, R. Genetic expression for monoterpenes in clones of *Pinus sylvestris* grown on different sites. *Scand. J. For. Res.*, 1988, **3**, 25–36.
8. Regimbal, J.-M. & Collin, G. Essential oil analysis of Balsam fir *Abies balsamea* (L.) Mill. *J. Essent. Oil Res.*, 1994, **6**, 5/6, 229–238.
9. Dunlop, P. J., Bignell, C. M., Jackson, J. F. & Hibbert, D. B. Chemometric analysis of gas chromatographic data of oils from *Eucalyptus* species. *Chem. Int. Lab. Syst.*, 1995, **30**, 1, 59–67.
10. Hiltunen, R. & Laakso, I. Gas chromatographic analysis and biogenetic relationships of monoterpene enantiomers in Scots pine and juniper needle oils. *Flavour Fragr. J.*, 1995, **10**, 203–210.

MÄNNI JA KUUSE OKKAÕLI KEMOMEETRILINE ANALÜÜS

Anne ORAV, Tiiu KAILAS ja Mihkel KOEL

Eesti männi ja kuuse okkaõli gaasikromatograafilise analüüsi andmete töötlemine peakomponentide meetodil näitas, et keskkonnatingimuste mõju terpeensete ühendite sisalduse muutumisele okastes on statistiliselt mittemärgatav. Mono-terpeenipaaride vahelise korrelatsiooni koefitsiendid annavad informatsiooni nimetatud ühendite seotuse või mitteseotuse kohta nende moodustumisel biosünteesi käigus.

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REFERENCES

1. Orav, A., Kailas, T., Lääm, A. & Koel, M. Gas chromatographic analysis of the monoterpene fraction of Estonian needle oils. *Annals of the Estonian Academy of Sciences*, 1995, 34(1), 149-157.
2. Orav, A., Kailas, T. & Lääm, A. Analysis of terpene composition of conifer oils by simultaneous distillation-extraction, gas chromatography and gas chromatography-mass spectrometry. *Chemosphere*, 1998, 43, 341-352.
3. Rudolf, E. Chromatographic studies in the genus *Picea* (Pinaceae). *Acta Bot. Fenn.*, 1967, 48, 4-82.
4. Javanan, S. Über die Terpenhydroxyen bei *Pinus sylvestris* L. *Ann. Bot. Fenn.*, 1968, 37, 1-12.
5. Hillman, R. Q. Variation, inheritance and chemical inter-relationships of monoterpenes in *Pinus*. *Canad. Jour. Bot.*, 1970, 48, 1-24.
6. Jantun, R. & Niemi, J. E. Correlational monoterpenoid variation in natural populations of *Pinus sylvestris* Sweden. *Scand. J. For. Res.*, 1988, 1, 82-91.
7. Jantun, R. & Niemi, J. E. Genetic expression for monoterpenes in clones of *Pinus sylvestris* grown in different sites. *Scand. J. For. Res.*, 1988, 3, 22-30.
8. Ragnafel, L. M. & Collin, G. Essential oil analysis of balsam fir (*Abies balsamea* (L.) Mill.). *J. Essent. Oil Res.*, 1994, 6, 365-370.
9. Dunlop, R. B., Wright, C. M., Jackson, J. R. & Roberts, D. B. Chromatographic analysis of the chromatographic data of six from *Pinus* species. *Can. Jour. Bot.*, 1953, 30, 1-32.
10. Hillman, R. & Laitio, J. Gas chromatographic analysis and biological relationship of monoterpenoid constituents in *Scots pine* and *European spruce*. *Ann. Bot. Fenn.*, 1965, 34, 205-210.