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ENANTIOMERS OF MONOTERPENIC HYDROCARBONS IN ESSENTIAL OILS FROM *JUNIPERUS COMMUNIS*

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Key Word Index—*Juniperus communis*; Cupressaceae; essential oils; needles; berries; wood; chiral analysis; monoterpene hydrocarbons; enantiomer separation.

Abstract—An investigation of the chiral and achiral composition of monoterpenic hydrocarbons in juniper oils has been undertaken. Samples were collected from different locations in Poland in two seasons (spring and autumn) and included needles, berries and wood. Surprisingly, large variations in the monoterpenic hydrocarbon compositions were observed. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Monoterpenes are components of essential oils produced and accumulated in large amounts by plants from certain families, including the Labiatae, Pinaceae, Cupressaceae, Umbelliferae and Rutaceae. Their composition can be useful in genetic and chemotaxonomic studies of coniferous species [1, 2]. The first reports concerning the enantiomeric separations of monoterpenes in essential oils were published in the early nineties. These studies were concerned with the elaboration of simple analytical methods adequate for chiral separations of these complex natural product mixtures [3, 4]. The enantiomeric composition of certain monoterpenes in essential oils has been the subject of several studies; differences between the relative amounts of enantiomers of some monoterpenes existing between species [5–7], as well as within one, have thus been reported [8].

The chemical (but not enantiomeric) composition of essential oils from juniper berries and needles have been studied by several authors [9–12]. Apart from our preliminary investigations concerning the chromatographic separation and chiral recognition of terpenic hydrocarbons in commercially available juniper oils [13], to our knowledge, more detailed studies have not yet appeared in the literature. It is already clear that differences in the chiral composition of monoterpenes depends on the origin of raw material. The present study concerns essential oils from *Juniperus communis* carefully harvested and extracted under controlled laboratory conditions. We were interested in learning if, and to what extent, the place of growing, the season of harvesting and the morphological part of one plant species influences the composition (chiral and achiral) of the constituent monoterpenes.

RESULTS AND DISCUSSION

Achiral analysis of juniper oils

The sites where most of the studied plant material was collected (except for the French sample) are shown in Fig. 1. The achiral composition of the monoterpenic hydrocarbons in the oils from these locations is presented in Table 1. Large variations in the composition of monoterpenes occur between individuals, as well as between the different morphological parts of a single specimen. The most variable compounds are sabinene, α-pinene and myrcene. A correlation between the relative amounts of α-pinene in needles and in wood can be observed in a given plant, though it is very different between individuals.

The amounts of sabinene are very low in all samples from the northern part of Poland (sites A–C, Fig. 1). In the rest of the samples (except D2) the sabinene content is much lower in berries than in either needles or wood. The large differences in the percentages of sabinene (0.3–42.5%) for needle samples from very close locations (D1 and D2) does not seem to confirm

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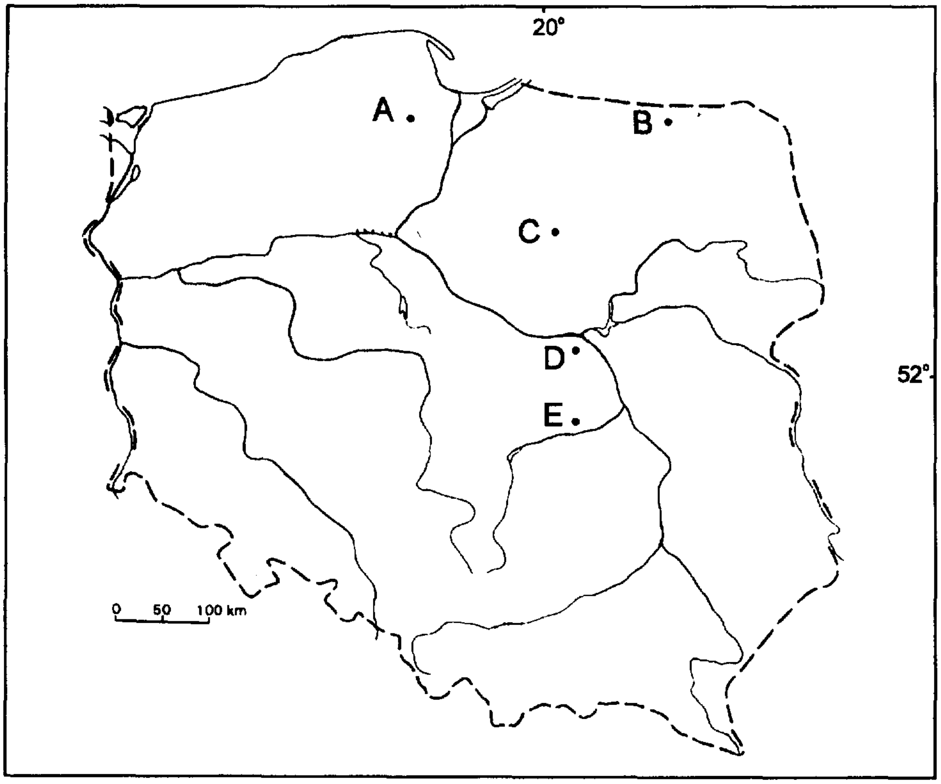

Fig. 1. Locations of juniper samples harvested in Poland.

the observations of Vernin *et al.* [9] and Baerheim Svendsen *et al.* [12], who both connected high amounts of sabinene with highland (above 500 m) growing juniper, since all but one of our samples were collected well below 500 m. Furthermore, a relatively low percentage of α-pinene in the low-growing samples studied here is not in agreement with the above cited suggestions.

The highest concentration of myrcene is observed in samples from Kampinos National Park (site D). The content of myrcene is consistently higher in berries than in needles and wood for a given specimen. Differences in the composition of monoterpenes in berries taken from one specimen (location A) harvested in autumn and spring were not observed, even though they were green (unripe) in autumn and black (ripe) in spring. In contrast, there were increasing contents of limonene (1.5–6.7%) and decreasing contents of mycrene (9.5–4.0%) and β-phellandrene (19.1–4.9%) in needles samples between autumn and spring.

Chiral analysis of juniper oils

Of the chiral compounds observed in the investigated oils, sabinene and β-pinene exist only in one enantiomeric form, sabinene as the dextrorotary (+)-form and β-pinene as the laevorotatory (−)-form. Because the amount of camphene was very low, in most cases below 1%, we do not present its enantiomeric ratio because these measurements are outside the limits of experimental error. The enantiomeric form of β-phellandrene also was not determined because of the lack of an appropriate standard. Figure 2 presents the enantiomeric excess of (*R*)-(+)-α-pinene which is calculated from:

$$EE(\%) = \frac{R-S}{R+S} 100$$

Large variations in the enantiomeric composition of α-pinene are observed between samples. In all samples from the northern part of Poland (A–C), (+)-α-pinene prevails at comparable levels, varying from 33 to 62%. Almost no difference between samples harvested in autumn and in spring is observed (A1 and A2). In two samples (E and F) collected from very distant locations (Poland and France), (−)-α-pinene dominates. Surprisingly, the most pronounced differences occur between samples from the same site D, situated in the central part of Poland. In essential oils from berries from site D, excesses of either the (+)- or (−)-enantiomer, as well as racemic compositions of α-pinene, are observed. The differences in the enantiomeric excess of α-pinene within plant D1′ (between needles and berries) was very pronounced. Interestingly, a similar differentiation was reported by Sjödin *et al.* [8] in *Pinus sylvestris*. The large variations in the chiral and achiral composition of monterpenes observed here may thus be influenced by the atypical place of growing for *J. communis*, namely swampy ground.

Table 1. Relative content of main components (%) of monoterpenic hydrocarbons in investigated juniper essential oils*

Sample	α-Pinene	Camphene	β-Pinene	Sabinene	3-Carene	Myrcene	α-Terpinene	Limonene	β-Phellandrene	γ-Terpinene	*p*-Cymene	Terpinolene
A1-n	72.2	0.7	2.9	2.6	2.6	4.0	0.6	6.7	4.9	0.3	1.0	0.8
A1-b	79.2	0.4	3.0	0.5	0.4	12.2	0.0	2.9	0.7	0.0	0.7	0.0
A2-n	63.8	0.9	2.1	0.9	1.8	9.5	0.0	1.5	19.1	0.0	0.0	0.0
A2-b	78.5	0.7	4.4	0.0	0.0	14.1	0.1	1.4	0.0	0.0	0.0	0.0
A2-w	63.7	1.8	2.2	0.6	10.5	5.2	0.0	3.1	7.9	0.0	1.4	3.1
B-n	89.7	0.4	2.9	0.7	0.4	3.2	0.0	1.3	0.6	0.1	0.0	0.7
B-w	90.0	0.8	2.6	0.4	0.8	2.6	0.0	1.3	0.5	0.1	0.0	0.8
C-n	79.5	0.6	2.5	1.8	1.6	3.9	0.0	4.3	5.6	0.2	0.0	0.0
C-w	72.1	1.6	2.9	1.3	5.8	2.0	0.0	8.0	3.8	0.0	3.0	0.0
D1-b	56.1	0.2	1.6	6.0	0.0	21.5	0.5	9.8	0.5	0.9	0.6	1.1
D1-n	36.8	0.2	1.3	33.1	0.2	4.4	1.3	15.8	0.8	2.2	1.2	1.8
D1-w	47.2	0.3	2.1	18.4	5.8	3.5	2.2	10.8	1.0	3.5	2.8	2.1
D1′-n	54.6	0.3	1.6	24.6	0.7	4.9	1.6	5.3	0.6	2.7	1.0	1.9
D1′-b	45.1	0.3	2.3	0.7	0.1	46.3	0.0	2.3	0.5	0.1	0.2	0.8
D1″-n	31.7	0.2	1.2	42.5	3.3	5.7	2.4	3.7	0.5	4.2	1.0	2.4
D1″-b	45.0	0.3	2.0	15.8	0.1	26.4	1.3	2.5	0.6	2.5	1.0	1.9
D2-b	55.6	0.2	2.1	10.4	0.5	26.0	0.5	1.8	0.5	1.2	0.4	0.9
D2-n	89.4	0.8	2.8	0.3	0.2	3.0	0.1	1.1	0.5	0.1	0.1	0.6
E-b	70.2	0.4	3.6	7.3	0.6	12.7	1.1	2.1	0.6	1.9	0.6	0.0
E-n	29.8	0.9	2.6	25.7	0.0	6.5	1.5	7.5	5.1	11.8	1.6	2.5
F-b	80.4	0.5	5.3	0.0	0.0	9.9	0.0	1.2	0.7	0.0	0.1	0.4
F-n	27.8	0.2	1.7	32.7	0.0	6.6	6.3	4.0	1.2	10.7	1.0	4.4
F-w	27.3	0.2	1.7	37.9	0.5	3.2	5.7	2.9	0.9	10.1	3.0	3.0

n = needles, b = berries, w = wood.

*Monoterpenic hydrocarbons together constituted 70–85% of the distilled essential oils.

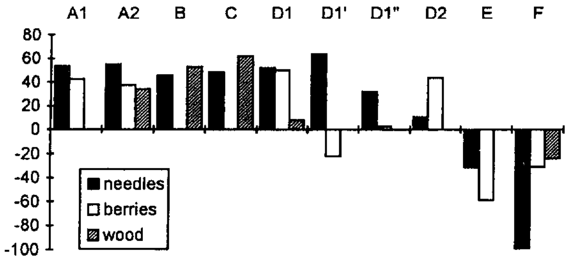

Fig. 2. Enantiomeric excess (%) of (*R*)-(+)-α-pinene in juniper oils.

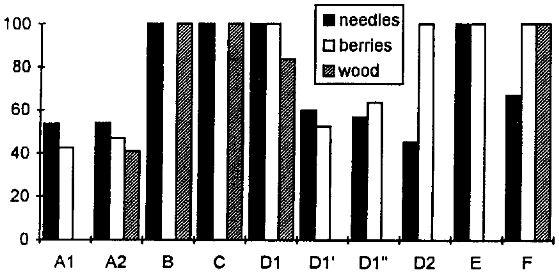

Fig. 3. Enantiomeric excess (%) of (*R*)-(+)-limonene in juniper oils.

The enantiomeric excess of (*R*)-(−)-limonene is presented in Fig. 3. In all samples, there is a large excess of (+)-limonene over its (−)-antipode, which seems to be a characteristic of the Rutaceae, while for essential oils from the Pinaceae (−)-limonene is most abundant [3, 8, 14].

Of all the investigated chiral monoterpenes identified in the juniper oils studied, the most different in enantiomeric ratio was α-pinene. The same observations was made by Sjödin *et al.* [8]. The other chiral monoterpenes appear as one or in large predominance of the other enantiomer. We did not observe significant differences in the composition of monoterpenic hydrocarbons (either chiral or achiral) between the two seasons (autumn and spring) or between green and black berries, while, according to Vernin *et al.* [9], the distinction between ripe and unripe berries is significant. This may be because the samples of green and black berries they studied were collected at the same time but perhaps from different individuals. In our case, the green berries were collected in autumn and once again after ripeness in spring from the same plant specimen. Because such significant differences in composition of monoterpenes occur between juniper plants from the same location it is therefore important to note whether the sample was obtained from one or more plants.

It is clear that more detailed studies addressing the genetic control and more specific ecological conditions are necessary in order to elucidate the factors governing the occurrence and composition of monoterpenic hydrocarbons in *J. communis*.

EXPERIMENTAL

Plant material. Plant material was collected from nine *J. communis* L. bushes (all samples except one from Poland) and identified in the Gdańsk Department of Botany. Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy (Medical University of Gdańsk, Poland) International Herbarium Index GDMA. The French sample came from southern Provance in the vicinity of Barreme (elevation above sea level over 500 m). Samples were collected from a Juniper bush growing in the valley of the river Asse. All Polish samples were located from below 500 m above sea level (Fig. 1). A full description of Polish sampling sites where our materials were collected is presented in Table 2. All investigated juniper specimens were *ca* 10 years old in full vitality and represented shrubby forms. Essential oils were obtained by steam dist. from freshly crushed berries as well as from freshly cut needles and wood.

GC and GC-MS analysis. Chromatographic studies were performed using an instrument equipped with FID. The column employed for achiral analysis was a WCOT CP-Wax 52 CB (Chrompack) fused silica capillary (30 m × 0.53 mm i.d., film thickness 1.0 μm). All chiral analyses were done using a glass column (4 m × 4 mm i.d.), filled with Chromosorb W NAW

Table 2. Characteristics of locations where juniper samples analysed were harvested

Samples	Location	Date of harvesting	Other species on sites
A	Piechowice	A1 October 1994 berries were green A2 March 1995 berries were black	Woodland with pine, birch and alder
B	Węgorzewo	October 1994	Woodland in pine forest
C	Mława	October 1994	Mixed forest periphery
D1	Kampinos Nat. Park Strict plant reserve 'Niepust'	October 1995	Swamp overgrown in 30% with alder, birch and pine
D2	Kampinos Nat. Park Young forest	October 1995	Pine 30–40 years, individually birch, oak and aspen
E	Świdno	October 1994	Mixed forest periphery; birch and pine
F	Barreme (France)	October 1995	Frangula, buckthorn, rowan

coated with an 0.8 M sol. of native α-cyclodextrin dissolved in a formamide medium. The complete procedure for prepn of the chiral packing has been described earlier [13]. Capillary column conditions: Ar at 10 kPa, oven temp. 60°, inj. temp. 200°; detector temp. 250°. Chiral α-cyclodextrin column conditions: Ar at 40 ml min^{-1}, oven temp. 35°, inj. temp. 200°; detector temp. 250°. For confirmation, monoterpenes were also identified by GC-MS using a mass-selective detector equipped with a WCOT HP-Innowax fused silica capillary (60 m × 0.32 mm i.d. × film thickness 0.50 μm).

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REFERENCES

1. Esteban, I., Bergmann, F., Gregorius, H.-R. and Huhtinen, O., *Silvae Genetica*, 1976, **25**, 59.
2. Fady, B., Arbez, M. and Marpeau, A., *Trees*, 1992, **6**, 162.
3. Ochocka, R., Sybilska, D., Asztemborska, M., Kowalczyk, J. and Goronowicz, J., *Journal of Chromatography*, 1991, **543**, 171.
4. König, W. A., *Gas Chromatographic Enantiomer Separation with Modified Cyclodextrins*. Hüthig, Heidelberg, 1991.
5. Ravid, U., Putievsky, E. and Katzir, I., *Flavour and Fragrance Journal*, 1994, **9**, 139.
6. König, W. A., Krüger, A., Icheln, D. and Runge, T., *Journal of High Resolution Chromatography*, 1992, **15**, 184.
7. Wang, X., Jia, C. and Wan, H., *Journal of Chromatographic Science*, 1995, **33**, 22.
8. Sjödin, K., Persson, M., Borg-Karlson, A.-K. and Norin, T., *Phytochemistry*, 1996, **41**, 439.
9. Vernin, G., Boniface, C., Metzger, J., Ghiglione, C., Hammoud, A., Suon, K., Fraisse, D. and Parkanyi, C., *Phytochemistry*, 1988, **27**, 1061.
10. Gelsomini, L., Vidrich, V., Fusi, P. and Michelozzi, M., *Journal of High Resolution Chromatography and Chromatography Communications*, 1988, **11**, 218.
11. Analytical Methods Committee, *Analyst*, 1984, **109**, 1343.
12. Baerheim Svendsen, A., Scheffer, J. J. C. and Looman, A., *Science Pharmaceutical*, 1985, **53**, 159.
13. Sybilska, D., Asztemborska, M., Kowalczyk, J., Ochocka, R., Osscini, L. and Perez, G., *Journal of Chromatography, Section A*, 1994, **659**, 389.
14. Holm, Y., Laakso, I. and Hiltunen, R., in *25th International Symposium on Essential Oils, Grasse, France*, Proceedings, Association des Ingénieurs et Techniciens de la Parfumerie, Grasse, 1994, abstracts, p. 57.