

Enantiomeric composition of terpenic hydrocarbons in essential oils from *Juniperus communis* L.

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ABSTRACT

α -Cyclodextrin complexation under appropriate conditions of partition gas chromatography has been applied for chiral discrimination of the hydrocarbon fraction of juniper oils of various origins. The essential oils under investigation were obtained from crushed juniper berries and leaves cultivated on plantations in Austria, Italy and Poland. It was found that the main component of the hydrocarbon fraction of almost all the juniper oils under investigation was a laevorotatory enantiomer, *S*(-)- α -pinene, and that the ratio of its *S* to *R* enantiomer may vary greatly (from 5 to 1) depending on the origin of the material, *i.e.* the part of the plant (berries or leaves), and the place of its cultivation.

INTRODUCTION

The chemical composition of essential oils of juniper has been the subject of numerous studies [1–6]. A comparison between the chemical composition of juniper oil and an alcoholic extract of juniper berries has also been recently reported [7]. It has been stated that their main characteristic component is α -pinene with a wide range of other monoterpene hydrocarbons, sesquiterpene hydrocarbons and some oxygenated compounds.

Many of the terpenes used therapeutically or as flavour and fragrance materials are chiral compounds, and thus they can be present in

natural mixtures in one or two enantiomeric forms and in various proportions. Furthermore, it is known that the stereospecificity of drug action is related to the molecular asymmetry of the component receptors of the human body. As a consequence, the diastereomers and enantiomers of chiral drugs have significantly different biological activities. This very important problem has not been explored sufficiently as yet, mainly because of the lack of analytical tools suitable to monitor the contents of enantiomers.

In this matter we have previously found that, by using α -cyclodextrin (α -CD) under appropriate conditions of classical partition gas chromatography (GC), very efficient separations of α -pinene, β -pinene and camphene into enantiomers can be achieved [8,9]. Attempts have also

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been made to develop this method for chiral recognition of hydrocarbons (mentioned above) and limonene, when they are present in more complex artificial mixtures or some natural ones, *i.e.* silver fir needle and pine needle oils [10].

Another method has recently been initiated by König and co-workers [11–13] and subsequently by Armstrong and co-workers [14,15], who applied some molten β -cyclodextrin derivatives in capillary gas chromatography.

Of the two ways mentioned above, only the first one makes possible operations on a micro-preparative scale, which may be useful for identification purposes and eventually for further physico-chemical measurements.

The current paper reports our attempts to apply this option using α -CD in a dissolved state as the stationary phase for the study of juniper oils of various origins: juniper berry oil, juniper leaf oil and juniper oil from leaves and berries derived from various European countries. The structural formulae of some chiral constituents of juniper berry oils currently under investigation are presented in Fig. 1.

Juniper oil displays definite antibiotic properties. It inhibits the growth of *Sarcina lutea*, *Bacillus mesentericus*, *Bacillus subtilis*, *Micrococcus pyogenes* var. *aureus*, *Serratia marcescens* and *Escherichia coli* and very strongly inhibits the growth of *Proteus vulgaris* [16].

In the 1930s, juniper oil in combination with anise oil and cajeput oil was applied in the treatment and prevention of cholera [17]. It has

been shown that juniper oil displays a strong activity against intestine worms during investigation both *in vitro* and *in vivo* [18]. The oil also has a strong repellent effect on the Egyptian mosquito (*Aedes aegypti*) [19].

The oil has also been used for therapeutic purposes in renal diseases and static and venous oedema.

Juniper berries are widely used in the manufacture of spices, perfumes and pharmaceutical products. The beverage industry is one of the main users of the berries' distillates. Differences in the quality of juniper berries and isolates on the market are commonly imputed to variation in the raw material.

To our knowledge, chromatographic separation and direct chiral recognition of terpenic hydrocarbons in juniper berry oil have not previously been attempted.

EXPERIMENTAL

Plant material

Berries and leaves from plants of *Juniperus communis* L. from Italy, Austria and from Poland were sampled.

The essential oils (samples I–III) were obtained from crushed juniper berries cultivated on plantations in:

- (1) Central Poland (Herbapol Enterprise, Łódź, Poland) (sample I).
- (2) The Tyrol in Austria (Brüder Unterweger, Thal-Assling, Austria) (sample II).
- (3) Central Italy (Primavera srL, Pisa, Italy) (sample III).

Sample IV was obtained from crushed juniper berries with leaves cultivated on a plantation in the north of Italy (Laboratori Italiani Vaillant, Milan, Italy). Sample V was produced from crushed juniper leaves obtained on a plantation in the Tyrol in Austria (Brüder Unterweger).

All investigated essential oils were obtained by steam distillation.

Reagents

α -CD was supplied by Chinoïn (Budapest, Hungary). Chromosorb W NAW (0.18–0.25 mm) for GC was from Johns-Manville, USA. All

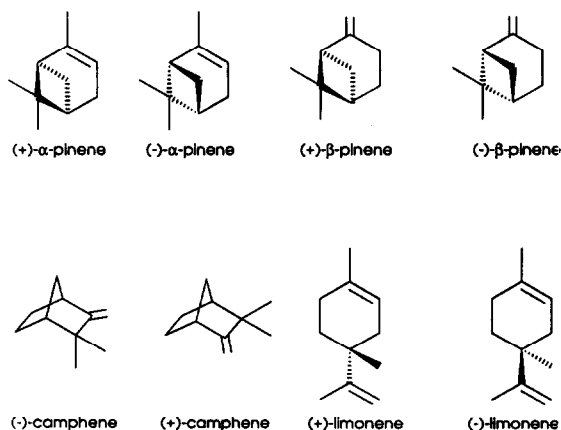


Fig. 1. Structural formulae of some investigated compounds.

other materials were of analytical reagent grade and were used without further purification.

Apparatus and procedure

Chromatographic studies were performed using a Hewlett-Packard Model 5890 gas chromatograph equipped with a dual flame ionization detector. The peak areas and retention times were measured by means of a Hewlett-Packard 3396 integrator.

The columns were as follows. Column A (achiral): HP-1 (Hewlett-Packard) cross-linked methylsilicone (non-polar) fused-silica capillary, 30 m × 0.53 mm I.D. Column B (chiral with α -CD): glass column, 2 m × 4 mm I.D., filled with Chromosorb W NAW coated with a 0.8 molal (*m*) solution of α -CD in formamide medium.

Packing of column B was prepared according to the following procedure. An aqueous homogeneous solution containing α -CD (0.6 g), formamide (3.4 g) and LiNO₃ (0.4 g) was deposited on 15 g of Chromosorb W NAW. The resulting slurry was shaken for about 10 min and the excess water was then slowly evaporated at 40°C/20 mmHg (1 mmHg = 133.322 Pa). The column was conditioned at 40°C for about 8 h.

Unfortunately, columns containing α -CD, although very efficient in chiral separations of terpenes, are not as stable as those containing β -CD and γ -CD. Until now their stabilization has been approached in two ways:

- (1) Swedish workers successfully applied a wetting procedure of carrier gas [20].
- (2) We used LiNO₃ for stabilization [21].

The role of water and LiNO₃ has not been clarified. It may involve structural changes in α -CD or it may be a simple solubilization process.

RESULTS AND DISCUSSION

Chemical composition

The main topic of the present study has been limited to the chiral recognition of monoterpene hydrocarbons in juniper oils. However, in order to keep our results compatible with the earlier achiral determinations, we used not only the chiral (B) column prepared by ourselves, but also a commercial achiral capillary column (A). The latter enabled us to estimate the contributions of the particular fractions in oils, including the fraction in which we were interested.

Table I gives the contents of the main hydrocarbons in all investigated samples determined by us using HP-1 capillary column A in comparison with the results published previously by other authors [1–7]. The referenced results concern exclusively oils obtained from berries.

Although the results collected in Table I were obtained in different laboratories and concern different samples from various manufacturers, they seem to be relatively similar, at least those dealing with the main components. In fact, the main hydrocarbon component of four of the investigated oils (I, II, III and V) is α -pinene. An interesting exception is oil IV derived from crushed berries and leaves cultivated in Italy, whose principal component is limonene.

Worthy of attention is also oil II (produced

TABLE I

CONTENTS OF THE MAIN MONOTERPENE HYDROCARBONS IN THE ESSENTIAL OILS OF JUNIPER DETERMINED ON CAPILLARY COLUMN HP-1 (A) IN COMPARISON WITH EARLIER LITERATURE DATA

Compound	Our results (%)					Reference (%)				
	I	II	III	IV	V	7	4	5	6	1
α -Pinene	35.6	45.7	39.2	10.3	37.0	20.0	26.0	38.4	35.1	18.7
Camphene	1.2	0.5	0.5	5.2	0.8	0.2	0.2	–	0.3	0.2
β -Pinene, sabinene	13.1	8.9	7.7	9.5	18.4	2.8	10.5	11.0	6.9	6.2
Limonene	11.8	5.2	4.5	15.5	8.1	8.7	3.8	2.5	3.7	2.0
Myrcene	0.7	21.5	13.1	0.5	3.5	8.5	9.0	11.7	9.5	1.8

from berries in the Tyrol), which is very rich in terpenic hydrocarbons; the data in Table I indicate that it contains about twice as much hydrocarbons as each of the other oils.

Enantiomeric composition

Generally accepted opinion suggests that the enantiomeric composition of terpenes depends on the plant of origin. This suggestion has recently been confirmed in our investigations [10]. We have found significant differences between the enantiomeric composition of chiral hydrocarbons in pine needle oil and fir needle oil, both from Poland, *i.e.* the main components of fir needle oil (overall *ca.* 76%) are laevorotatory monoterpene hydrocarbons, while in pine needle oil dextrorotatory hydrocarbons predominate.

As the next step we have undertaken a study of other factors. The present paper reports our investigations using the same kind of plant, namely juniper, but varying the parameters place in Europe of juniper cultivation and part of the plant (berries or leaves) used as the starting material for manufacturing the oil. The direct separations of enantiomers of the monoterpene fraction of the five investigated oils on an α -CD column (B) are presented in Fig. 2. In Table II the corresponding semiquantitative values are presented.

Recognition of enantiomers by α -CD is truly remarkable. Some data on enantioselectivity factors estimated from artificial mixture given in Table III support this statement. However, α -CD seems to be a very poor differentiator of $C_{10}H_{16}$ isomers of other types (pinenes, limonene, camphene, terpinenes and others). As a consequence, a well-separated enantiomer of one compound may be superimposed by an enantiomer of another, as it is seen in Fig. 2 for (+)-camphene and sabinene, (+)-limonene and *p*-cymene, (-)- α -pinene and (-)- β -pinene.

Further studies on optimization of the procedure to achieve complete separation of isomers and enantiomers in a single chromatographic process are in progress. At present to achieve this goal we have applied another method, *i.e.* the rationalization of the data obtained on chiral column B with those of achiral capillary column

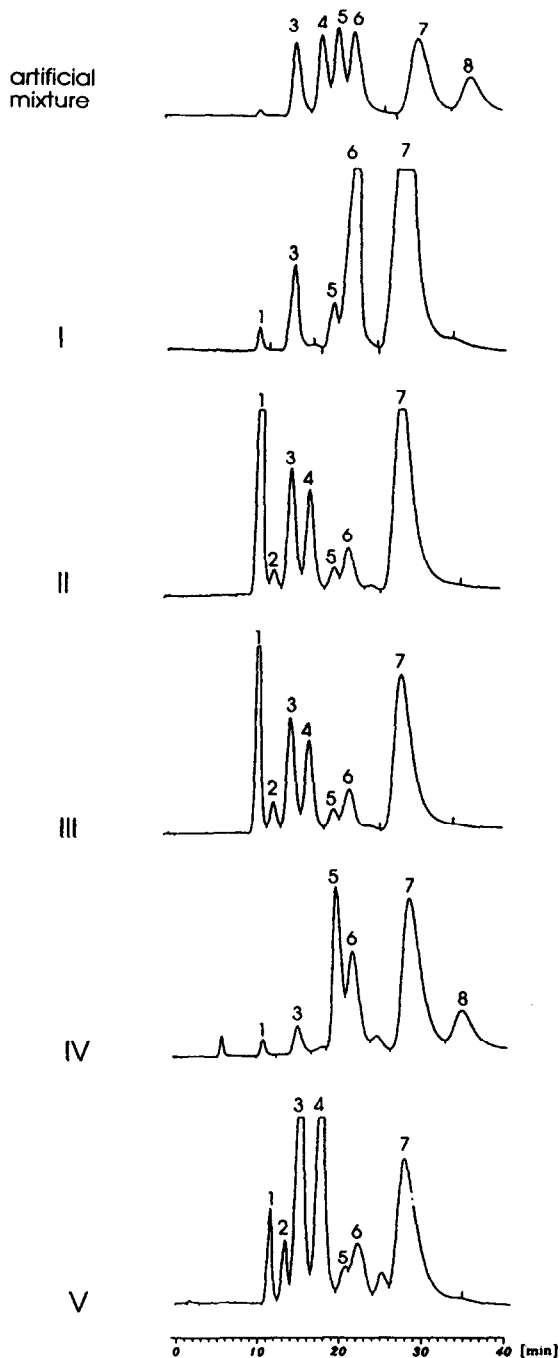


Fig. 2. Chromatograms of all investigated juniper oils and an artificial mixture obtained on chiral column with α -CD (B). Column temperature: 30°C. Flow-rate: 40 ml/min argon. Peaks: 1 = myrcene; 2 = α -terpinene; 3 = (+)- α -pinene; 4 = (+)-camphene, sabinene; 5 = (-)-limonene, (+)- β -pinene; 6 = (+)-limonene, *p*-cymene; 7 = (-)- α -pinene, (-)- β -pinene; 8 = (-)-camphene.

TABLE II

CONTENTS OF THE MAIN COMPONENTS OF THE MONOTERPENE FRACTION IN JUNIPER OILS DETERMINED ON A CHIRAL COLUMN (B) WITH α -CD

Compound	Contents (%)				
	I	II	III	IV	V
Myrcene	1.2	27.7	19.5	1.1	4.7
α -Terpinene	–	1.9	3.3	–	3.8
(+)- α -Pinene	7.3	11.4	14.3	3.6	22.9
(+)-Camphene, sabinene	0.5	9.9	12.4	0.5	25.3
(-)-Limonene	3.6	2.3	2.9	20.4	3.1
(+)-Limonene, <i>p</i> -cymene	28.7	5.6	7.5	16.5	8.2
(-)- α -Pinene, (-)- β -pinene	57.4	40.6	40.4	40.4	29.2
(-)-Camphene	1.0	–	–	13.4	–

A. In the evaluation of the contents of two compounds eluted together from the chiral column [e.g. (-)- α -pinene and (-)- β -pinene], the data from the achiral column are taken into account, assuming the monoterpene fraction to be 100%.

The final corrected results are given in Table IV.

It is seen that the ratio of *S*(-) to *R*(+) of α -pinene, although always greater than 1, varies greatly: from about 5 for Italian oil IV through 2.7 for Italian oil III to about 1 for the almost racemic composition of the oil produced from leaves in the Tyrol (V). In all the investigated oils, except IV, *R*(+)-limonene predominated, the ratio of *R*(+) to *S*(-) enantiomers varying

TABLE III

SEPARATION FACTORS (α) AND ORDER OF ELUTION OF ENANTIOMERS OF SOME MONOTERPENE HYDROCARBONS OBTAINED ON A CHIRAL COLUMN (C) WITH α -CD (0.8 *m*) AT 30°C

Compound	α	First peak eluted
α -Pinene	1.92	(+)
Camphene	1.93	(+)
Limonene	1.09	(-)
β -Pinene	1.45	(+)

TABLE IV

ENANTIOMERIC COMPOSITION OF THE MONOTERPENE FRACTION OF JUNIPER OILS CALCULATED BY RATIONALIZATION OF THE RESULTS OBTAINED ON A CAPILLARY COLUMN HP-1 (A) AND A CHIRAL COLUMN WITH α -CD (B)

Compound	Composition (%)				
	I	II	III	IV	V
(+)- α -Pinene	8	11	14	4	23
(-)- α -Pinene	38	38	38	20	24
(+)-Limonene	15	5	4	15	7
(-)-Limonene	3	2	2.5	20	3
(+)-Camphene	0.5	1	1	–	–
(-)-Camphene	1.0	–	–	12	1
(+)- β -Pinene			Traces		
(-)- β -Pinene	18	3	3	22	4
Sabinene	–	8	8	–	20
<i>p</i> -Cymene	10	1	1	1	5
Myrcene	1	25	18	1	1
α -Terpinene	–	1.5	2.5	–	2

from 5 for the Polish oil I to 1.6 for Italian oil III; in contrast to this in the Italian oil IV it is *S*(-)-limonene which predominates.

In almost all the oils camphene constitutes the minor component (1–2%), the exception being oil IV, in which a considerable quantity of camphene (greater than 10%) was found.

In all the samples only traces of *R*(+)- β -pinene were found.

In the Polish I and Italian IV oils considerable amounts of *S*(-)- β -pinene (18–22%) were found but no sabinene. The rest of the oils under investigation contain relatively small amounts of *S*(-)- β -pinene (3–4%) accompanied by a significant amount of sabinene, from 8 to 20%.

In summary, the method appointed to monitor the enantiomeric composition of terpenic hydrocarbon in essential oils, although still not ideal, has been elaborated. Its application to juniper oils reported here indicates that the problem of enantiomeric composition of terpenic hydrocarbons produced by plants, *i.e.* the search for the main factor responsible for the content of enantiomers, is very complicated and is still unsolved. Further studies are in progress.

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REFERENCES

- 1 N. Gelsomini, V. Vidrich, P. Fusi and M. Michelozzi, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 218.
- 2 H. Kallio and K. Jurger-Mannermaa, *J. Agric. Food Chem.*, 37 (1969) 1013.
- 3 D. Lamparsky and I. Klimes, *Parfum. Kosmet.*, 66 (1985) 553.
- 4 J.E. Karg, *Parfum. Kosmet.*, 62 (1981) 341.
- 5 M. Melagari, G. Vampa, A. Bianchi, A. Albasini, S. Benvenuti, M. Rinaldi and A. Provvigionato, *Atti Soc. Nat. Mat. di Modena*, 118 (1987) 75.
- 6 Analytical Methods Committee, *Analyst*, 109 (1984) 1343.
- 7 A. Proenca da Cunha and Odete L.R. Roque, *J. Ess. Oil. Res.*, 1 (1989) 15.
- 8 T. Kościelski, D. Sybilska and J. Jurczak, *J. Chromatogr.*, 280 (1983) 131.
- 9 D. Sybilska, J. Kowalczyk, M. Asztemborska, T. Stankiewicz and J. Jurczak, *J. Chromatogr.*, 543 (1991) 397.
- 10 R.J. Ochocka, D. Sybilska, M. Asztemborska, J. Kowalczyk and J. Goronowicz, *J. Chromatogr.*, 543 (1991) 171.
- 11 W.A. König, S. Lutz and G. Wenz, *Angew. Chem. Int. Ed. Engl.*, 27 (1988) 979.
- 12 W.A. König, R. Krebber, P. Evers and G. Bruhn, *J. High Resolut. Chromatogr.*, 13 (1990) 328.
- 13 W.A. König, A. Krüger, D. Icheln and T. Runge, *J. High Resolut. Chromatogr.*, 15 (1992) 184.
- 14 D.W. Armstrong, W. Li and J. Pitha, *Anal. Chem.*, 62 (1990) 217.
- 15 D.W. Armstrong, W. Li, C.D. Chang and J. Pitha, *Anal. Chem.*, 62 (1990) 914.
- 16 J.C. Maruzella and M.B. Lichtenstein, *J. Am. Pharm. Assoc.*, 45 (1956) 378.
- 17 J.W. Tomb, *J. Trop. Med. Hyg.*, 29 (1926) 210.
- 18 G. Valette, R. Cavier and J. Debelmas, *Ann. Pharm. Franc.*, 11 (1953) 649.
- 19 D. Kalemba, A. Kurowska, J. Góra, S. Łakota and A. Lis, *Pestycydy*, 2 (1991) 31.
- 20 M. Lindström, T. Norin and J. Roeraade, *J. Chromatogr.*, 513 (1990) 315.
- 21 D. Sybilska and J. Jurczak, *Carbohydr. Res.*, 192 (1989) 243.