ENANTIOMERIC COMPOSITION OF MONOTERPENE HYDROCARBONS FROM THE LIVERWORT *CONOCEPHALUM CONICUM*

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Abstract—Volatiles from the essential oil of the liverwort *Conocephalum conicum* were analysed. The chirality of the monoterpene hydrocarbons was studied by two-dimensional gas chromatography. All compounds except β-phellandrene showed high optical purity. For the identification of enantiomers, (+)-β-phellandrene and (−)-α-thujene were prepared from (+)-limonene and (+)-sabinene, respectively.

INTRODUCTION

Essential oils of liverworts have been studied for a long time. Many monoterpene hydrocarbons and oxygenated monoterpene derivatives have been found to be important constituents, together with sesquiterpene hydrocarbons, alcohols and lactones [1]. The essential oil of the thalloid liverwort *Conocephalum conicum* L. has been the object of several studies. In some of these studies, the chirality of isolated components has been reported. δ-Cadinene has been found to be the main component of the sesquiterpene fraction [2] and to occur in the form of the (−)-enantiomer [3]. Furthermore, a number of sesquiterpene lactones, which inhibit the plant growth in rice, have been isolated from this species by Asakawa and Takemoto [4].

It has been shown that the main constituent among the monoterpenes in *Conocephalum conicum* is sabinene [5]. Bornyl acetate is also present in a high concentration and is considered as the main source of the characteristic fragrant odour of this plant. No significant differences in the monoterpene fraction have been found between female and male gametophytes [6]. (−)-Sabinene and (−)-limonene were found in the first study of the chirality of monoterpenes from *Conocephalum conicum* [7]. We now report the enantiomeric composition of several other monoterpene hydrocarbons present in the essential oil of this species. Biosynthetic considerations based on chiral relationships between the monoterpene constituents are also presented.

RESULTS AND DISCUSSION

The monoterpene hydrocarbons that we found in the essential oil of *Conocephalum conicum* are listed in Table 1. The main component was sabinene, in agreement with previous reports [5, 6]. γ-Terpinene and α-terpinene were also present in significant proportions. The above study of the chirality of monoterpenes had been based on the isolation of each compound, followed by measure-

Table 1. Proportions and enantiomeric compositions of monoterpenes in volatiles from *Conocephalum conicum*

Compound*	Relative %	Enantiomeric composition in monoterpene fraction (% +/−)
α-Thujene	6	>95/<5
α-Pinene	1	2/98
Camphene	3	4/96
β-Pinene	2	<0.1/>99.9
Sabinene	37	<0.1/>99.9
Myrcene	3	non-chiral
α-Phellandrene	trace	†
α-Terpinene	8	non-chiral
Limonene	6	3/97
β-Phellandrene	2	31/69
p-Cymene	2	non-chiral
γ-Terpinene	15	non-chiral
Terpinolene	3	non-chiral

*The compounds are listed according to the retention order on a DB-WAX column.

†The content was too low to allow a determination of the enantiomeric composition.

ment of its optical rotation [7]. Using a new and highly sensitive two-dimensional GC technique we were able, without previous isolation, to determine the exact enantiomeric composition of most of the monoterpene hydrocarbons in a crude mixture, even the ones present in very low quantities.

Almost all of the monoterpenes showed high optical purity, the (−)-enantiomer prevailing (more than 96%, i.e. 92% e.e.). (+)-α-Thujene was an exception but on the other hand this compound is biosynthetically correlated

to (−)-sabinene. The possible presence of a small proportion of (−)-α-thujene could not be excluded, because we did not reach base-line separation of the two enantiomers with any chiral column available. One compound, β-phellandrene, showed a low optical purity [69% (−)-enantiomer, i.e. 38% e.e.]. This fact does not agree with the proposal by Suire et al. [7], that liverworts produce only pure enantiomers of chiral terpenoids.

Our present knowledge of the biosynthesis of mono-terpenes [8] indicates that the enzyme system of this liverwort is less complicated than those of higher plants. Croteau [9] has isolated two types of cyclases from sage leaves and suggested a scheme of biosynthesis of enantiomerically pure monoterpenes. Based on mechanistic studies, he has explained both the occurrence of (−)-β-pinene in very high optical purity in plants, and on the other hand the common presence of α-pinene, camphene and limonene as mixtures of enantiomers. Our results indicate that even in C. conicum the production of (−)-α- and (−)-β-pinene, (−)-limonene and (−)-camphene may be catalysed by Croteau's 'cyclase II' and proceed via α-terpinyl cation as an intermediate [9].

Croteau [9] has suggested a different pathway for the formation of sabinene and α-thujene. As for the chirality, he isolated a (+)-sabinene cyclase similar to cyclase II but having the opposite stereospecificity [9]. As we found (−)-sabinene in C. conicum, there may be a (−)-sabinene cyclase of the same stereospecificity as cyclase II operating in this liverwort.

The formation of (−)-β-phellandrene has been postulated to proceed via the same intermediate (α-terpinyl cation) as the formation of (−)-α- and (−)-β-pinene [10]. As we found both (+)- and (−)-β-phellandrene, there must be still another biosynthetic pathway operating in C. conicum. Safe conclusions regarding the types of cyclization enzymes acting in liverworts can be drawn only after detailed biosynthetic studies.

Our results are a contribution to the knowledge of the enantiomeric compositions of monoterpenes in plants, which provide useful information for biosynthetic considerations. We have also shown that C. conicum is a source of several enantiomerically pure monoterpenes. Our methods for the preparation of chiral standards of the ubiquitous monoterpenes β-phellandrene and α-thujene should be useful for various phytochemical investigations.

EXPERIMENTAL

Sample preparation. The liverwort of Conocephalum conicum was collected in April 1990 at Hrubá Skála u Trutnova, Czechoslovakia. The plant material was washed with cold water to remove the soil. After drying at room temp. the entire plants (150 g) were crushed in a mixer together with H_2O (1 l). The material was subjected to a 1 hr hydrodistillation using a Clevenger-type apparatus. The volatiles were trapped in pentane (2 ml) and directly chromatographed on a silica gel column (15 g), with pentane (20 ml) as the eluent.

Analysing technique. The volatile fraction obtained after distillation was a mixture of monoterpenes and sesquiterpenes. Our attention was focused on the monoterpene hydrocarbons. The monoterpenes were identified by GC-MS with a HP-5 capillary column (5% phenyl methyl silicone).

2D-GC was used for the chiral analysis. Two Varian 3400 gas chromatographs were coupled in series. The first one was equipped with a DB-WAX column (bonded polyethylene glycol), 30 m, 0.25 mm i.d. (J&W Scientific). The second gas chromatograph contained two chiral columns of the type Cyclodex B (permethylated β-cyclodextrin), 30 m, 0.25 mm i.d. (J&W Scientific). For the separation of the enantiomers of α-thujene, a Lipodex E (dipentyl butyryl γ-cyclodextrin) column, 30 m, 0.25 mm i.d. (Macherey–Nagel) was installed in the same GC instrument. The monoterpene hydrocarbons were first separated on the non-chiral column and then let into the chiral column using a Valco 4-port valve (0.010 port diameter, 1/32″ fitting), controlled by He under pressure. Two chiral columns were used in parallel to avoid overlapping of peaks of (+)-camphene and (+)-sabinene. A splitless injection technique and flame ionization detectors were used. The carrier gas (He) flow was 0.82 ml min^{-1} in the non-chiral column and 1.24–1.30 ml min^{-1} in the chiral columns. A temp. prog. from 65° (11 min) to 130° (20° min^{-1}) on the DB-WAX column, and isothermal separation
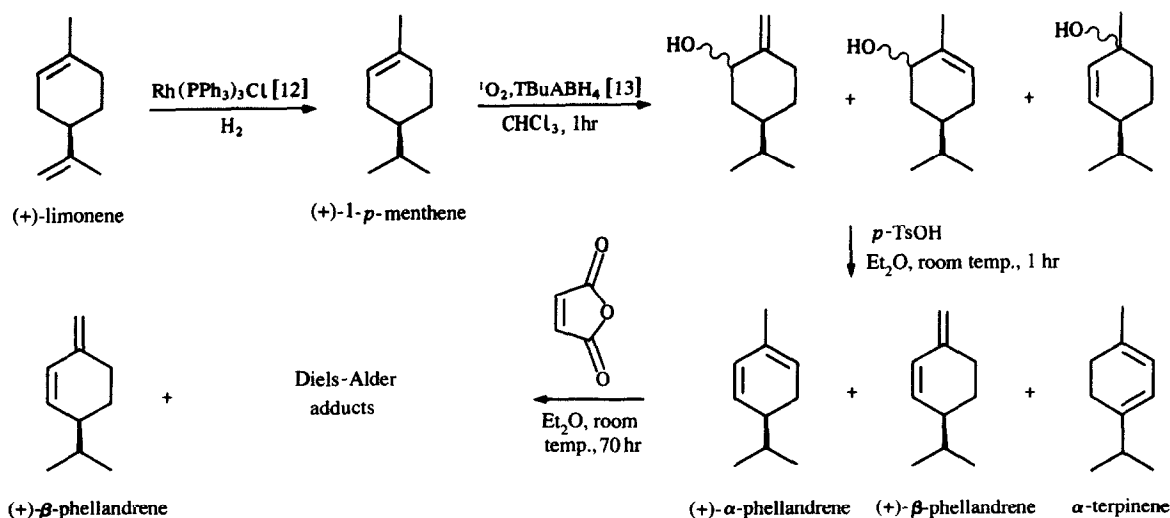
Scheme 1.

at 55° on the Cyclodex B column were used. The analysis of α-thujene on the Lipodex E column was performed at 29° isothermally. The elution order of the enantiomeric pairs was as follows (1st peak/2nd peak): (−/+)-α-pinene, (−/+)-camphene, (+/−)-sabinene, (+/−)-β-pinene, (−/+)-limonene, and (−/+)-β-phellandrene (Cyclodex B column), (−/+)-α-thujene (Lipodex E column). The detailed description of our 2D-GC technique has been presented elsewhere [11]. To verify the structures of (+)-β-phellandrene and (−)-α-thujene, ¹H NMR spectra were recorded at 400 MHz.

Reference chemicals. The majority of the standards were commercial chemicals of the highest optical purity available. The (+)-β-pinene and racemic β-phellandrene samples were gifts from Firmenich SA. (+)-β-Phellandrene and (−)-α-thujene were prepared in our laboratory on a small scale by the following procedures:

(+)-β-*Phellandrene.* (+)-Limonene was hydrogenated according to the literature [12] using chlorotris(triphenylphosphine)-rhodium (I) as a catalyst in tetrahydrofuran (Scheme 1). The (+)-1-*p*-menthene obtained was photooxidized in the presence of tetrabutylammonium borohydride (TBuABH₄) according to ref. [13]. The crude reaction mixture was subjected to medium-pressure liquid chromatography on silica gel. The fractions containing monooxygenated products were combined and dehydrated under acidic catalysis with *p*-toluenesulphonic acid in Et₂O. The resulting mixture of α- and β-phellandrene (containing small amounts of α-terpinene and *p*-cymene), was treated with maleic anhydride [14, 15]. After chromatographic removal of the Diels–Alder adducts from the unchanged hydrocarbons (β-phellandrene and *p*-cymene), (+)-β-phellandrene was obtained in a low yield. The structure of the final product was verified by ¹H NMR and mass spectroscopy and by measuring the optical rotation ([α]_D, +48°, CDCl₃; c 0.15). The literature data for the optical rotation of β-phellandrene differ within a wide range from +20.8° [16] to −74.4° [17]. As only a small amount of the pure compound was obtained (3 mg), the measurement of its optical rotation was on the accuracy limit of the polarimeter (Perkin–Elmer 241). The optical purity, as checked by GC on a Cyclodex B column, was 99.5%.
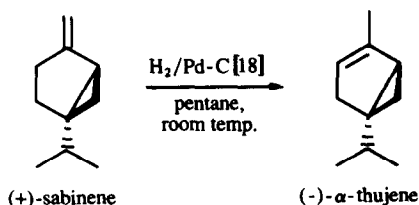
(−)-α-*Thujene.* (−)-α-Thujene was prepared (Scheme 2) by the isomerization of (+)-sabinene according to the procedure described in ref. [18]. Pd on charcoal in pentane suspension was activated with hydrogen and used as a catalyst for the isomerization of (+)-sabinene. (−)-α-Thujene was formed besides a small amount of hydrogenated material. The structure of the product was confirmed by comparison of its mass spectrum with that reported in MS library.

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Scheme 2.