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IDENTIFICATION OF (+)-β-CARYOPHYLLENE IN ESSENTIAL OILS OF LIVERWORTS BY ENANTIOSELECTIVE GAS CHROMATOGRAPHY

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Abstract—A combination of capillary columns with conventional and chiral stationary phases in a double-oven gas chromatograph (2D-GC) was employed to investigate the enantiomeric composition of β-caryophyllene in several liverworts. By comparison with a synthetic standard of racemic β-caryophyllene, enantioselective gas chromatography using heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin as a chiral stationary phase revealed the presence of up to 85% (+)-β-caryophyllene in some specimens of *Pellia endiviifolia* and *P. epiphylla*.

INTRODUCTION

The occurrence of sesquiterpene enantiomers with opposite configuration in the essential oils of liverworts as compared to higher plants was first described by Huneck and Klein [1] in *Scapania undulata* (L.) and was consistently reported in subsequent investigations of other liverwort species [2]. However, with the applied methods (optical rotation or CD measurements) it is difficult to determine the exact composition of an enantiomeric mixture. After the introduction of highly selective hydrophobic cyclodextrin derivatives as chiral stationary phases [3], the enantiomeric proportion of sesquiterpene hydrocarbons could be determined very precisely by capillary GC [4]. So far no results have been reported about the occurrence of the unusual (+)-enantiomer of β-caryophyllene in liverworts. We have now carried out an investigation of the enantiomeric composition of β-caryophyllene in the volatiles of liverworts and some higher plants.

RESULTS AND DISCUSSION

For the investigation of enantiomeric compositions by enantioselective gas chromatography it is necessary to determine the elution order and R_ts of the individual enantiomers by injecting an enantiomeric mixture of known composition. Procedures for the total synthesis of β-caryophyllene have been elaborated by Corey *et al.* [5] and by Ohtsuka *et al.* [6]. In a more convenient procedure racemic β-caryophyllene can be prepared from α-humulene according to Greenwood *et al.* [7]. The product obtained together with an excess of 4,11,11-trimethyl-8-methylenetricyclo[7, 2, $0^{1,9}$, $0^{2,4}$]undecane (see Fig. 1 for structure) and α-humulene was purified by preparative GC (2.2% yield of nearly pure (±)-β-caryophyllene). The resolution of the enantiomers on a capillary column containing heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin [8] is demonstrated in Fig. 1; the elution order was determined by comparison with commercial (−)-β-caryophyllene.

β-Caryophyllene, although a common constituent of the essential oils of many liverworts, is usually only a minor component and is, therefore, not always eluted as a single peak even from a capillary column with high separation efficiency. Thus, the enantiomeric composition can not securely be determined by comparing R_ts or co-injections of authentic standards. For complex mixtures, as in the case of essential oils, 2D-GC is the preferred method [9]. After separating the components of the mixture on a conventional capillary column (a non-polar CpSil 5 capillary was used in this study) the interesting portion of the chromatogram (the β-caryophyllene peak) is transferred to a second capillary column containing the chiral stationary phase, which is kept at an appropriate temperature in an adjacent second gas chromatographic oven. The enantiomers are resolved on this column and quantified by electronic peak integration. Correct peak assignment is proved by co-injection of the racemic standard as demonstrated in Fig. 2. Before, the presence of β-caryophyllene in the investigated essential oils was verified by gas chromatographic-mass spectrometric analysis.

As shown in Table 1, the enantiomeric composition of β-caryophyllene of a given liverwort depends on the site of collection. The largest proportion of the unusal (+)-β-caryophyllene is found in *Pellia endiviifolia*; however, smaller amounts are also detected in *P. epiphylla* and

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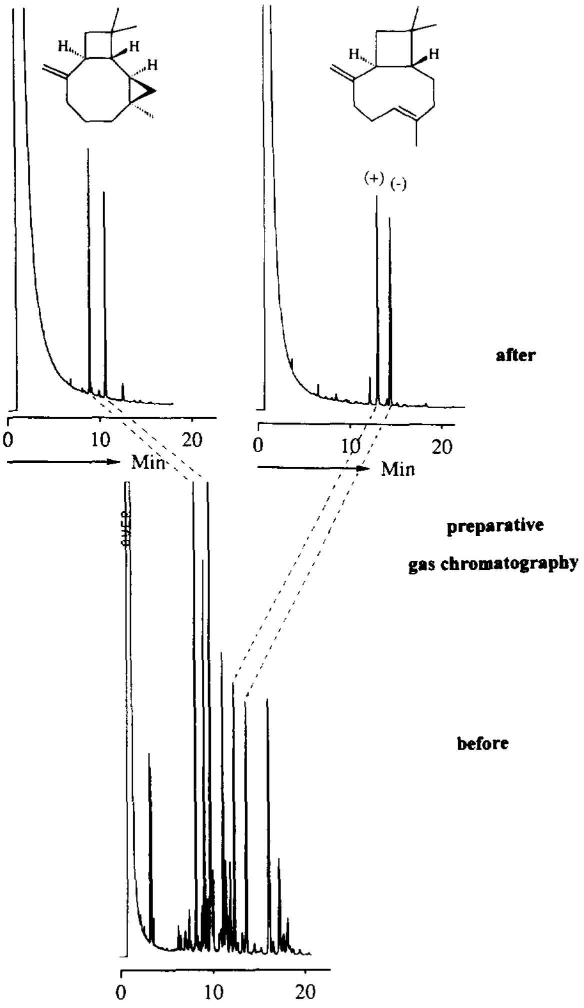

Fig. 1. GC enantiomer separation of the synthetic hydrocarbon mixture before (lower part) and after (upper part) preparative GC isolation of β-caryophyllene (right, (−)-enantiomer is shown) and its tricyclic isomer (left). 25 m Capillary column containing 20% heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin in polysiloxane OV 1701 (w/w) at 100°; carrier gas H_2 at 50 kPa.

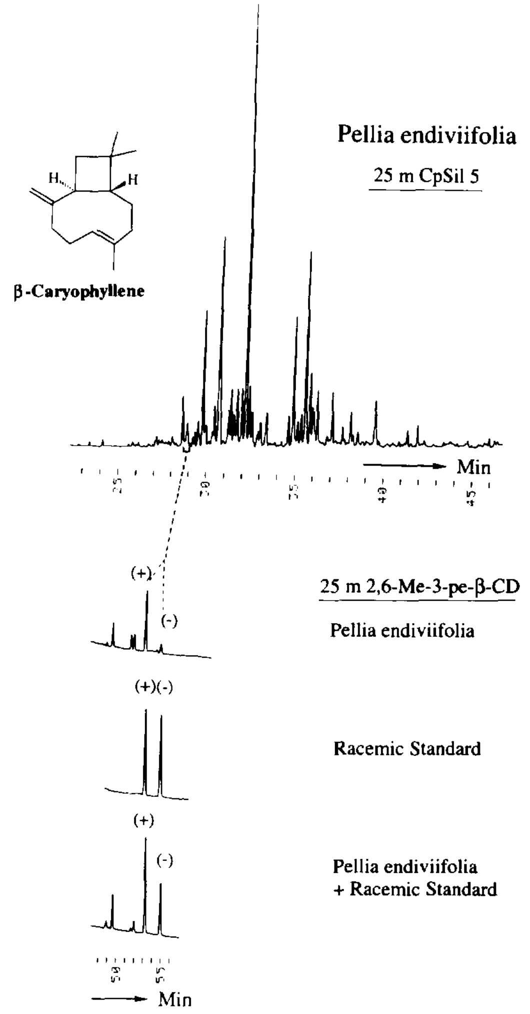

Fig. 2. Two-dimensional gas chromatographic investigation of β-caryophyllene (structure of (−)-enantiomer is shown) in the essential oil of the liverwort *Pellia endiviifolia*. Upper part: 25 m CpSil 5 capillary column, 50°, temp. programme 1° $\min^{-1}$ to 200°. Lower part: partial chromatograms of the β-caryophyllene fraction after transfer to a 25 m capillary column with 50% heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin in polysiloxane OV 1701 (w/w) at 110°. Racemic β-caryophyllene and a mixture of it with the essential oil are shown below.

Metzgeria conjugata. Pure (−)-β-caryophyllene was identified in the liverworts *Fossombronia alaskana*, *F. pusilla*, *Trichocolea tomentella* and *Ptilidium pulcherrimum* as well as in hop oil, black pepper oil, clove leaf oil, copaiva balsam oil and opoponax oil. In *Cedrela odorata* oil, where some other sesquiterpene hydrocarbons were found to be mixtures of enantiomers [10], the presence of a trace of (+)-β-caryophyllene could not be excluded.

β-Caryophyllene is one of the few sesquiterpene hydrocarbons frequently present in the essential oil of liverworts where the unusal (+)-enantiomer has not yet been detected. (+)-β-Caryophyllene has only once been described as a natural product thus far. Pigulevskii and Borovkov [11] isolated β-caryophyllene with a positive optical rotation from the essential oil of *Libanotis transcaucasica*.

Although enantiomeric mixtures of monoterpene hydrocarbons are commonly found in essential oils [12], definite proof of the presence of both enantiomers of sesquiterpene hydrocarbons in the same plant has only recently been given [4]. The co-occurrence of both enantiomers raises the question of its biogenetic origin. It may be an indication of two different biogenetic pathways. While the enantiomer in excess may be generated enzymatically, an additional *racemic* portion of the same compound may originate from rearrangement processes of achiral precursors. Thus, δ-elemene in essential oils is always found as a racemic mixture [4], probably formed (as an artefact) from achiral germacrene C by Cope-rearrangement, while optically active γ-elemene may be formed by dehydration of elemol and as a racemate by Cope-rearrangement of achiral germacrene B. In the case

Table 1. Enantiomeric composition of *trans*-β-caryophyllene in various essential oils

Liverwort/Origin	(+)-Enantiomer (%)	(−)-Enantiomer (%)
Pellia epiphylla/Trittau/Germany	20.21	79.71
Pellia epiphylla/Arbersee/Germany	11.29	88.71
Pellia endiviif./Grasse/France	39.70	60.30
Pellia endiviif./Hamburg/Germany	85.61	14.39
Metzgeria conjugata/Allgäu/Germany	10.86	89.14
Trichocolea tomentella/Allgäu/Germany	—	100
Ptilidium pulcherrimum/Arbersee/Germany	—	100
Fossombronia alaskana/unknown origin	—	100
Fossombronia pusilla/unknown origin	—	100

of β-caryophyllene, a humulyl cation precursor for both α-humulene and (−)-β-caryophyllene is suggested by labelling studies [13]. This may explain the co-occurrence of both sesquiterpenes in many essential oils. However, the mechanism for the generation of (+)-β-caryophyllene and other 'unusual' enantiomers of sesquiterpene hydrocarbons is unknown.

EXPERIMENTAL

Plant materials and essential oil preparation. Liverworts were collected at the sites given in Table 1, except for *Fossombronia alaskana* and *F. pusilla*, the hydrocarbon fractions of which were a gift of Professor H. Becker, Universität Saarbrücken. The essential oils were prepared by steam distillation of aqueous homogenates of the green plants. Essential oils of higher plants were gifts of Mr K.-D. Protzen, Paul Kaders AG, Hamburg.

Synthesis of racemic β-*caryophyllene.* The procedure of ref. [7] was used. It was found to be important that in the final reductive step with $LiAlH_4$ the reagent is completely dissolved in tetrahydrofuran.

Preparative GC. A 2 m (5.1 mm i.d.) stainless steel column (Silcosteel, Amchro) coated with 6% of a 1:1 mixture of polysiloxane PS-086 and octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-cyclodextrin on Chromosorb W-HP (Merck) was used with He as carrier gas at a flow rate of 240 ml min^{-1}. About 2 mg of the synthetic mixture of sesquiterpene hydrocarbons (see above) in 60 μl of hexane was injected at 100°, and the temp. increased at 2° min^{-1} to 150°. β-Caryophyllene eluted after about 12 min. The fractions were collected in Teflon tubes cooled in a bath of liquid N_2 as described earlier. The total yield of β-caryophyllene was 2.2% (*ca* 95% chemical purity after prep. GC) with respect to α-humulene and afforded mass spectra and ^1H NMR spectra identical with those of commercial β-caryophyllene (Fluka).

Capillary GC. Capillary columns with cyclodextrin derivatives were prepared as described earlier [12].

Two-dimensional gas chromatography. The essential oil samples were injected on a 25 m (0.25 mm i.d.) capillary column containing non-polar CpSil 5 (Chrompack) in a Siemens Sichromat 2 gas chromatograph at 50° and programmed at a rate of 1°/min to 200°. Automatic sample transfer was performed after 34.05 min (the R_t of β-caryophyllene) to a 25 m capillary column containing heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (50% in polysiloxane OV1701, w/w) which was kept isothermally at 110°. The chromatograms from both columns were recorded with a two-channel Merck-Hitachi model 2500 integrator. H_2 at an entrance pressure of 80 kPa for the CpSil 5 capillary and 65 kPa for the cyclodextrin capillary was used as a carrier gas.

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