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THE SESQUITERPENE CONSTITUENTS OF THE LIVERWORT *PREISSIA QUADRATA*

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Key Word Index—*Preissia quadrata*; Hepaticae, liverwort; germacrene C; (+)-germacrene D; 2D-GC; cyclodextrin derivatives.

Abstract—The labile sesquiterpene hydrocarbon germacrene C is the main constituent of a chemotype of the liverworth *Preissia quadrata* collected in Southern Germany. Its temperature dependent rearrangement to racemic δ-elemene is observed by capillary gas chromatography using cyclodextrins as chiral stationary phases. In addition, the rarely found *ent*-sesquiterpenes (+)-germacrene D, (+)-*trans*-β-caryophyllene, (+)-β-caryophylleneoxide, (+)-cubebol and racemic alismol [1β,5α-guaia-6,10(15)-dien-4-ol] were identified as major constituents using spectroscopic methods and enantioselective gas chromatography. Minor constituents are (+)-α-copaene, (+)-δ-selinene, (−)-δ-cadinene, germacrene B and (−)-cascarilladiene (eudesma-5,7-diene). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Germacrene C (**1**) was first isolated as the main constituent of dried fruits of *Kadsura japonica* by Morikawa and Hirose [1] and characterized as a precursor of δ-elemene (**2**). It readily rearranged in a *Cope*-rearrangement at 100°C in solution to δ-elemene. Under slightly acidic conditions (silica gel at room temp.) it was smoothly converted to δ-selinene (**3**) and 4(15),6-selinadiene (**4**). Alismol (**5**) was first described as a constituent of the rhizomes of *Alisma plantago-aquatica* var. *orientale* by Oshima *et al.* [2]. Only recently the structure of **5** was revised by Yoshikawa *et al.* [3] with respect to the relative stereochemistry at C-5. These authors also suggested that **5**, and some derivatives of **5**, are degradation products of germacrene C formed during processing of the plant material.

RESULTS AND DISCUSSION

The investigated sample of *Preissia quadrata* [4, 5] was found near Ulm (southern Germany) and submitted to hydrodistillation. The volatiles were collected in hexane and investigated by GC-mass spectrometry. The occurrence of large amounts of germacrene C in the steam distillate of *P. quadrata* indicates an unexpected stability of **1**. However, capillary GC using a cyclodextrin derivative as a chiral stationary phase clearly revealed the thermal rearrangement of **1** to racemic **2** when the temperature of the injection port was raised from 200° to 280° as illustrated in Fig. 1.

Although considerable conversion of **1** to **2** in the inlet system of the gas chromatograph could be observed at temperatures above 240°C, it was possible to isolate germacrene C by preparative GC [6] and to perform NMR measurements and rearrangement experiments. A large peak of δ-elemene and a high ion current level over more than 10 min with high relative intensities of the ions *m/z* 121 and 136 (typical for δ-elemene) in the total ion current chromatogram indicated partial conversion of germacrene C during GC-mass spectrometry by *Cope*-rearrangement. As already observed for germacrenes A and B, which also thermally rearrange to β- and γ-elemene, respectively, the mass spectra of the germacrene precursors and their rearrangement products are practically identical.

The stereochemistry of the other sesquiterpenes was determined by two-dimensional capillary gas chromatography (2D-GC) using a non-polar polysiloxane (CpSil 5) capillary column for pre-separation and heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin as a chiral stationary phase in the main column as described previously [7]. β-Caryophyllene (**6**) (100% ee), β-caryophylleneoxide (**7**) (100% ee) and germacrene D (**8**) (86% ee) are present as the unusual (+)-enantiomers. While (+)-**6** was recently detected as a constituent of several liverworts [8] the latter two, though present in several other liverworts [9], were not found before in hepaticae as the unusual enantiomers. (+)-Germacrene D has been described as a constituent of *Solidago altissima* L. [10] and of the soft coral

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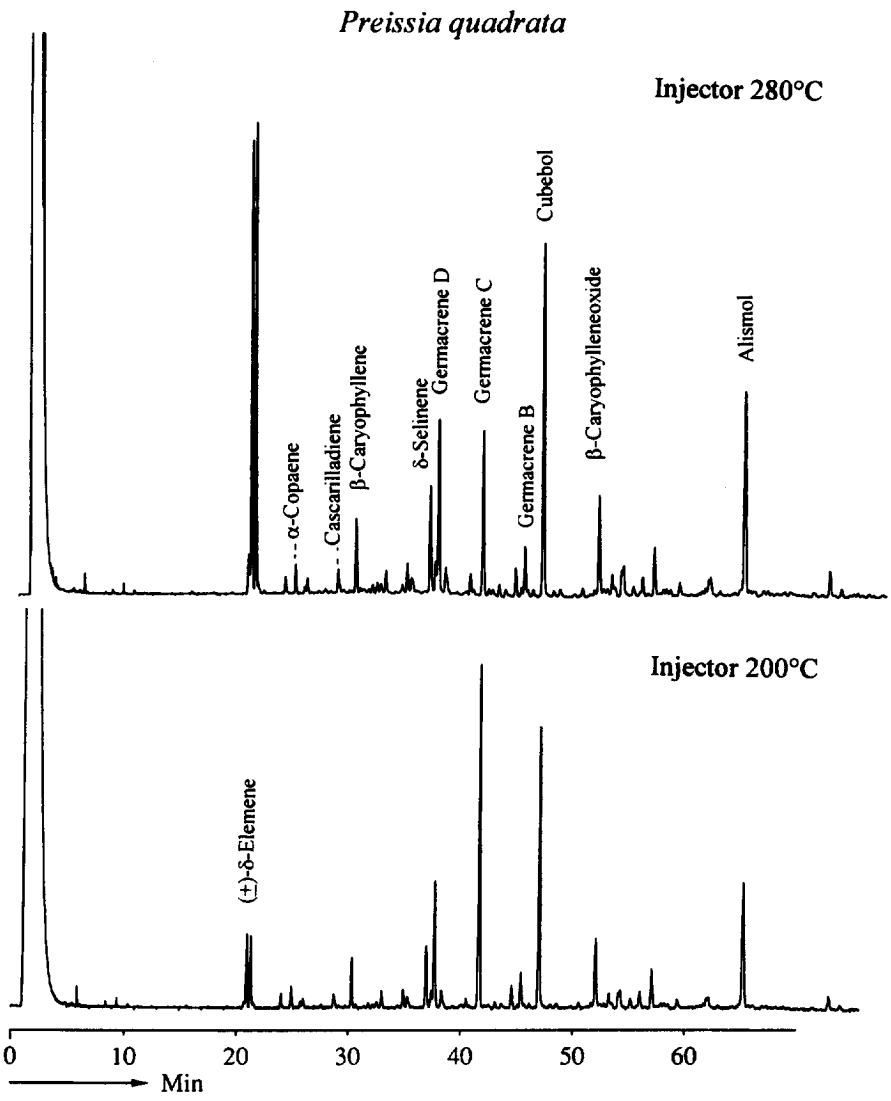


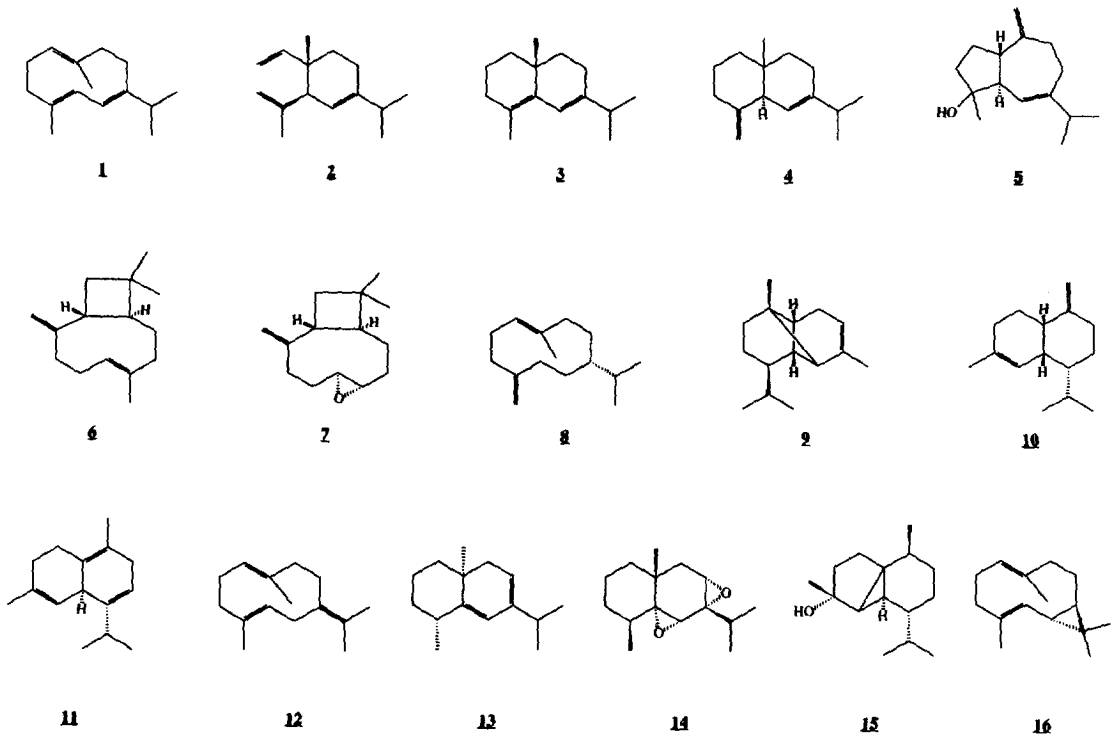
Fig. 1. Gas chromatographic separation of the hydrodistillate of *P. quadrata* at an injector temperature of 200° (bottom) and 280° (top). 25 m Fused silica capillary column with octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (50% in polysiloxane OV1701, w/w). Column temp. 70°C, temp. progr. 1°C min^{-1}. The relative amount of germacrene C is greatly diminished at 280°C, while the relative amount of racemic δ-elemene is increased.

Sinularia mayi [11]. Germacrene D with varying enantiomeric composition is also the main sesquiterpene component of *S. canadensis* and *S. gigantea* growing ubiquitously in central Europe. Both enantiomers were isolated from the hydrodistillates by preparative enantioselective GC and characterized by NMR investigations (Bülow, N. and König, W. A., unpublished results).

In addition, racemic alismol (**5**) was identified as a major constituent of *P. quadrata*. The fact that **5** was found as a racemate is indeed an indication that it is formed from germacrene C as already suggested by Yoshikawa *et al.* [3] in the case of *A. orientale*. A complete set of ^{1}H and ^{13}C NMR data for **5** is given in the experimental part. The assignment of the ^{13}C peaks was achieved on the base of a COLOC experiment and is reversed for C-1 and C-5 as compared to ref. [3].

As minor components (+)-α-copaene (**9**), (+)-δ-selinene (**3**), (−)-γ-amorphene (**10**), (−)-δ-cadinene (**11**), germacrene B (**12**) and (−)-cascarilladiene (**13**) were identified by comparison of their mass spectra and retention indices with authentic reference material and by enantioselective GC (Fig. 2).

Cascarilladiene with so far unknown optical rotation is a major constituent of cascarilla oil (*Croton eluteria* Bennett) [12]. Its structure was identified by Weyerstahl *et al.* [13]. This group also identified its absolute configuration by chemical correlation with the bis-epoxide (**14**) which had been found before by Bohlmann *et al.* [14] in *Liabum floribundum*. We isolated cascarilladiene from cascarilla oil by preparative GC and found a positive optical rotation. The specific optical rotation could not be determined because it was not possible to completely remove an impurity of (−)-



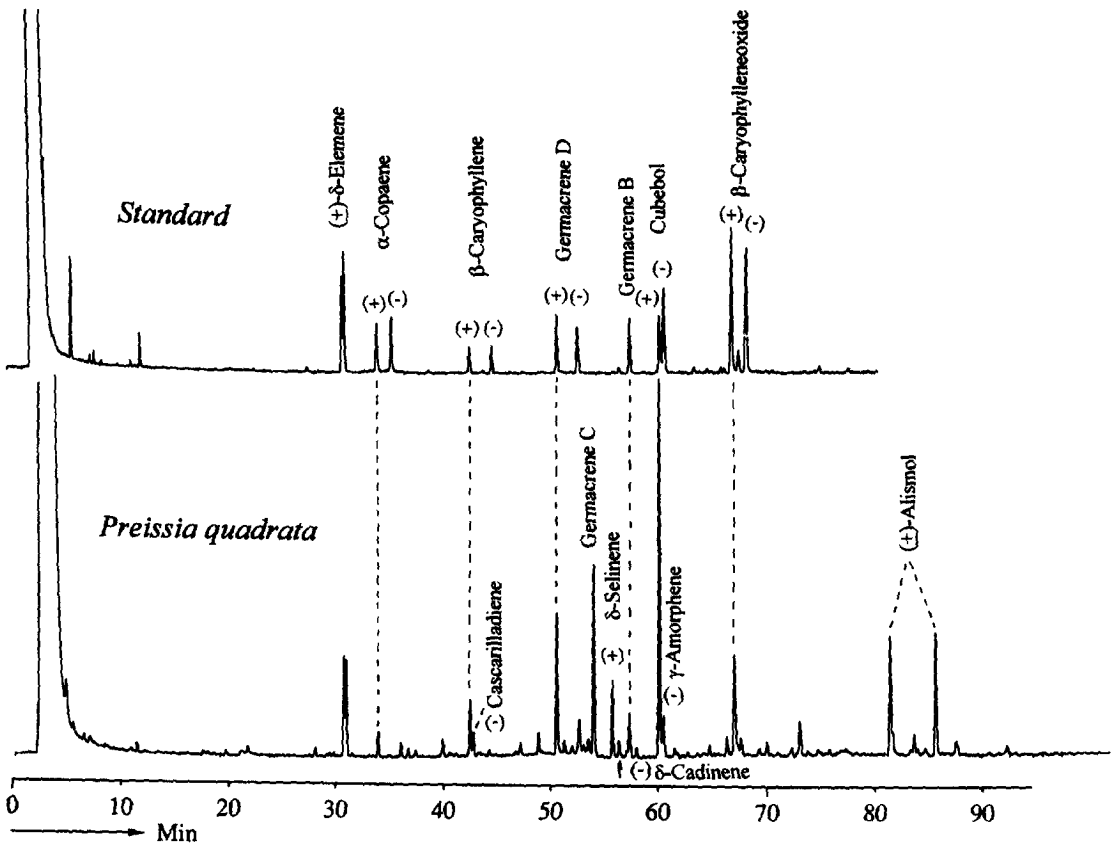


Fig. 2. Gas chromatographic separation of the hydrodistillate of *P. quadrata* (bottom) and a standard mixture of enantiomers (top). 25 m Fused silica capillary column with heptakis(6-*O*-t-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (50% in polysiloxane OV 1701, w/w). Column temp. 110°, 15 min. isothermal, then 1° min^{-1} to 160°.

β-caryophyllene, which elutes very close to cascarilladiene on all stationary phases available.

Another major constituent of *P. quadrata*, (+)-cubebol (**15**) (100% ee), was isolated before from a certain chemotype of the liverwort *Conocephalum conicum*, while the (−)-enantiomer was isolated from the essential oil of *Piper cubeba* (Melching, S. and König, W. A., unpublished results). (−)-Isolepidozene (**16**) and (+)-cubebol (**15**), but not germacrene C, were identified as main constituents in another sample of *P. quadrata* collected in Austria (Gauertal, Vorarlberg). Again, this indicates the existence of different chemical races as already observed for many other liverworts.

EXPERIMENTAL

Plant material. Preissia quadrata was collected in September 1995 at Jungingen, near Ulm (Germany) and identified by H. M. The collected liverwort is deposited in the Institut für Allgemeine Botanik, Universität Hamburg.

Hydrodistillation. The essential oil of *P. quadrata* was prepared by steam distillation (2 hr) of aq. homogenates of fresh and green plants using *n*-hexane as collection solvent. Because of the greatly differing weight the fresh material was not weighed.

Preparative GC. Isolation of **1**, **5** and **13** was performed by preparative GC on a Varian 1400 instrument, equipped with a stainless steel column (Silcosteel, Amchro) (2.05 m × 5.1 mm) with 6% octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin-PS-086 (1:1; w/w) on Chromosorb W-HP. He was used as carrier gas at a flow rate of 240 ml min^{-1}.

Two-dimensional gas chromatography. The essential oil samples were injected on a 25 m (0.25 mm i.d.) capillary column with dimethylpolysiloxane CpSil 5 (Chrompack) in a Siemens Sichromat 2 gas chromatograph at 50° and programmed at a rate of 3° min^{-1} to 200°. Sample transfer was performed after 36.48 min (the R_t of germacrene D), 34.93 (the R_t of β-caryophyllene), 33.96 min (the R_t of cascarilladiene), and respectively after 38.09 min (R_t of cubebol) 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° (germacrene D, β-caryophyllene and cascarilladiene) and, respectively, at 120° (cubebol). 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.

NMR spectroscopy. NMR measurements were performed with WM 400 (400 MHz) instrument (Bruker) using TMS as int. standard.

GC-MS. Electron impact (70 eV) GC-MS measurements were carried out on a Hewlett Packard HP 5890 gas chromatograph coupled to a VG Analytical VG 70-250S mass spectrometer.

Polarimetry. Optical rotation measurements were performed with a Perkin Elmer 241 polarimeter.

Germacrene C (**1**). ^{1}H NMR (C_6D_6): δ 6.34 (1H, *d*, *J* = 9.3 Hz), 5.31 (1H, *d*, *J* = 9.3 Hz), 4.87 (1H, *dd*, *J* = 11.2 Hz, *J* = 4.6 Hz), 1.54 (3H, *s*), 1.16 (3H, *s*), 1.05 (3H, *d*, *J* = 6.3 Hz), 1.03 (3H, *d*, *J* = 6.3 Hz).

Alismol (**5**). ^{1}H NMR ($CDCl_3$): δ 5.56 (1H, *s*), 4.76, 4.71 (each 1H, *br s*), 1.25 (3H, *s*), 1.00 (3H, *d*, *J* = 6.6 Hz), 0.99 (3H, *d*, *J* = 6.6 Hz). ^{13}C NMR ($CDCl_3$): δ 153.9 (C-10), 149.8 (C-7), 121.3 (C-6), 106.5 (C-15), 80.7 (C-4), 55.0 (C-5), 47.3 (C-1), 40.2 (C-3), 37.4 (C-11), 37.1 (C-9), 30.0 (C-8), 24.7 (C-2), 24.1 (C-14), 21.5, 21.3 (C-12 and C-13). MS (EI, 70 eV), *m/z* (rel. int.): 220 (8), 202 (35), 187 (31), 177 (20), 162 (41), 159 (75), 147 (35), 145 (39), 131 (60), 119 (90), 117 (41), 107 (40), 105 (64), 93 (51), 91 (89), 81 (27), 79 (48), 77 (43), 67 (30), 55 (33), 43 (100), 41 (84).

Cascarilladiene (**13**). ^{1}H NMR ($CDCl_3$): δ 5.61 (1H, *br s*), 5.32 (1H, *dm*, *J* = 6.4 Hz), 2.58 (1H, *m*), 2.25 (1H, *m*), 2.07 (1H, *br d*, *J* = 16.4 Hz), 1.86 (1H, *dd*, *J* = 1.64 Hz, *J* = 66 Hz), 1.15 (3H, *d*, *J* = 7.5 Hz), 1.01 (3H, *d*, *J* = 6.8 Hz), 1.00 (*d*, *J* = 6.8 Hz), 0.97 (3H, *s*). MS (EI, 70 eV), *m/z* (rel. int.): 204 (39), 189 (58), 161 (49), 147 (70), 136 (37), 133 (49), 121 (55), 119 (45), 105 (77), 93 (56), 91 (100), 79 (28), 77 (31), 65 (17), 55 (26), 43 (46), 41 (68).

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