Chirality Evaluation of 2-Heptanol and *Z*-4-Hepten-2-ol from Banana Fruit Using Multidimensional Gas Chromatography

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A polar fraction obtained from banana fruit pulp by high-vacuum distillation / solvent extraction (pentane-dichloromethane, 2 + 1) and subsequent liquid chromatography on silica gel was derivatized with isopropylisocyanate. Separation of enantiomers of heptan-2-ol and *Z*-4-hepten-2-ol was performed *via* their carbamates by means of multidimensional gas chromatography using an achiral (DB-5, column 1) and a chiral (XE-60, column 2) capillary column. The enantiomeric ratios of heptan-2-ol and *Z*-4-hepten-2-ol were determined to be (*R*)(−) 39% and (*S*)(+) 61% as well as (*R*)(+) 26% and (*S*)(−) 74%, respectively. The absolute configuration of optically enriched (*S*)(−)-*Z*-4-hepten-2-ol was determined from its (*R*)-2-phenylpropionic acid ester by ^{1}H NMR spectroscopy.

Introduction

The importance of the relationship between chirality and biological activity is well appreciated in pheromone and flavour perception. The determination of enantiomeric compositions and absolute configurations of biologically active compounds is highly warranted in pheromone chemistry [1] and flavour analysis [2].

Recently, the enantiomeric distribution of several chiral alkan-2-yl esters from banana fruit has been determined [3], but chirality evaluation of their alcohol moieties has not been carried out. This paper concerns the results of our studies on enantiodifferentiation of the major aliphatic secondary alcohols among banana volatiles, *i.e.* heptan-2-ol and *Z*-4-hepten-2-ol, by multidimensional gas chromatography (MDGC).

Materials and Methods

Materials

Heptan-2-ol, dodecanoic acid, (*R*)(−)-2-phenylpropionic acid and isopropylisocyanate were purchased from Aldrich, Steinheim. *Z*-4-hepten-2-ol was kindly provided by ORIL SA, Paris. Porcine pancreas lipase 7023 C was purchased from Röhm, Darmstadt. Fresh banana fruits were available from the local market. Optically pure (*S*)(+)-heptan-2-ol was kindly provided by D. Gerlach, Würzburg.

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Sample preparation

Banana fruits were stored at room temperature until optimum of ripeness. After removal of the skin, a total of 1.9 kg of diluted fruit pulp was obtained from 1.2 kg of total fruit after addition of 1 l distilled water and homogenization in a Braun blender. The pulp was subjected to high-vacuum distillation (45 °C, 0.1 mbar) [4] with subsequent solvent extraction (pentane-dichloromethane, 2 + 1) [5]. Prefractionation of the carefully concentrated distillation extract by liquid chromatography on silica gel with a pentane-diethyl ether gradient [6] led to two fractions. Fraction I (pentane-diethyl ether (9 + 1)) was discarded, fraction II (diethyl ether) was dried over Na_2SO_4, concentrated to 0.5 ml, and subjected to derivatization for MDGC analysis.

Derivatization with isopropylisocyanate

Fraction II was further concentrated in a conic vial under a stream of N_2 to approximately 0.1 ml and derivatized with 0.5 ml isopropylisocyanate (IPIC) (1.5 h, 100 °C). Excess of reagent was removed under a stream of N_2 and 0.5 ml dichloromethane were added for subsequent MDGC and MDGC-MS analysis.

Heptan-2-ol, (*S*)-heptan-2-ol, *Z*-4-hepten-2-ol, and (*S*)-*Z*-4-hepten-2-ol diluted in 0.2 ml dichloromethane each were transferred to the corresponding isopropylurethanes with 0.2 ml IPIC in the same way for elucidation of retention times and elution sequences.

Optical enrichment of Z-4-hepten-2-ol

According to the methodology of Gerlach *et al.* [7] for alkan-2-ols 5 mmol *Z*-4-hepten-2-ol were esterified with 5 mmol dodecanoic acid in 20 ml heptane (bidistilled) under lipase catalysis at 40 °C. After 48 h a yield of 53% (HRGC control, DB-5) was reached and the reaction stopped by filtration. After concentration *in vacuo* to 0.5 ml the remaining alcohol was separated by liquid chromatography (10 cm × 1 cm id; silica gel) with a pentane-diethyl ether gradient (fraction I, 50 ml pentane-diethyl ether (9 + 1), discarded; fraction II, 50 ml diethyl ether, dried over Na_2SO_4 and concentrated *in vacuo* to 0.5 ml). Ten µl of the concentrated eluate were subjected to derivatization for MDGC analysis, as described above, revealing an enantiomeric excess of 81.2%. After concentration to dryness (N_2) and dilution in 2 ml ethanol (abs.) evaluation of optical rotation value on a Perkin Elmer 241 MC polarimeter (589.5 nm) revealed the enrichment of the (−)-antipode. After concentration *in vacuo* the (−)-*Z*-4-hepten-2-ol was subjected to derivatization with (*R*)(−)-2-phenylpropionic acid for ^{1}H NMR spectroscopy.

Synthesis of Z-4-hepten-2-ol (R)-phenylpropionic acid esters

A 2 mmol equivalent portion of (*R*)(−)-2-phenylpropionic acid was transferred to the corresponding acid chloride with oxalyl chloride (5 min, 60 °C). Three times 1 ml benzene was added and distilled off leading to optically pure (*R*)-2-phenylpropionic acid chloride used for esterification of 1 mmol (*R,S*)-alcohol and (−)-alcohol, respectively (3 d, 60 °C). After concentration the solutions were stirred with 2 ml dioxan/water (1 + 1) and extracted with diethyl ether-benzene (7 + 3). The organic layers were treated with 1 N NaOH, washed with water until neutral reaction and dried over Na_2SO_4. Purification after concentration was achieved by TLC on silica gel (eluent, CH_2Cl_2). Purities: 100% (HRGC control, DB-5). ^{1}H NMR data and spectra are outlined in Table I and Fig. 3.

Multidimensional gas chromatography (MDGC). Multidimensional gas chromatography-mass spectrometry (MDGC-MS)

A double oven gas chromatograph Siemens Sichromat 2 with split injector (200 °C, 1 : 15) and FID on oven 1 as well as FID on oven 2 was used. Preseparation was achieved on a J&W DB-5 fused silica capillary column (30 m × 0.25 mm id; film thickness 0.25 µm). The temperature program was 80 °C–300 °C at 4 °C/min. A "live"-switching device [8] in oven 1 was used to perform effluent cuts on column 2 in oven 2 (Chrompack XE-60-S-VAL-S-A-PEA 50 m × 0.25 mm id; film thickness 0.12 µm). The temperature program was isothermal until start of cut at 90 °C and then from 90 °C–150 °C at 1 °C/min. At the end of column 2 a second modified "live-T" [9] was used for effluent splitting between FID and MS (Finnigan MAT 44; SS-200 data system; temperature of the ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.8 mA). He was used as carrier gas (inlet pressure, 3.0 bar; auxiliary pressures at live-T 1 and 2, 2.4 bar and 1.1 bar, respectively).

Results of qualitative analyses were verified by comparison of HRGC retention and mass spectral data with those of authentic reference substances. Quantitative HRGC determinations were carried out by peak area calculations on Shimadzu CR-6-A computing integrators, without consideration of calibration factors.

NMR spectral analysis

^{1}H NMR spectra were recorded on a Bruker spectrometer AC 200 (200-MHz). Samples were run in $CDCl_3$ using Me_4Si as internal standard.

Results and Discussion

A polar fraction obtained from banana fruit pulp by high-vacuum distillation / solvent extraction (pentane-dichlorometane, 2 + 1) and subsequent liquid chromatography on silica gel was derivatized with IPIC as auxiliary [10]. For the separation of enantiomers of heptan-2-ol and *Z*-4-hepten-2-ol *via* their carbamates multidimensional gas chromatography (MDGC) [11] using an achiral (DB-5, column 1) and chiral (XE-60, column 2) capillary column was employed. After preseparation of the IPIC derivatives of racemic and optically highly enriched reference compounds on the achiral DB-5 column, temperature program, cut times, and elution sequence were evaluated in order to optimize their HRGC separation on the chiral XE-60 capillary. The MDGC separations of IPIC derivatives of racemic reference compounds are represented in Fig. 1, those of derivatized substances from banana extract are out-

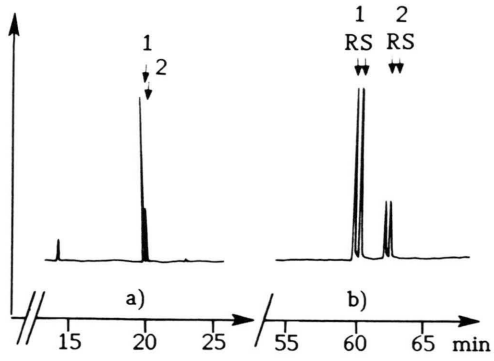

Fig. 1. MDGC separations of IPIC-derivatives of racemic reference compounds; (**1**) heptan-2-ol; (**2**) *Z*-4-hepten-2-ol; a) preseparation on DB-5; b) separation of diastereomers on XE-60; *cf.* experimental section for details.

lined in Fig. 2. The purity of the two peaks of IPIC derivatives obtained after cut was confirmed by mass spectrometry. The enantiomeric ratios of heptan-2-ol and *Z*-4-hepten-2-ol were determined to be (*R*)(−) 39% and (*S*)(+) 61% as well as (*R*)(+) 26% and (*S*)(−) 74%, respectively.

Since no information was available on the absolute configuration of *Z*-4-hepten-2-ol enantiomers in the literature, the (−)-antipode was obtained by lipase-catalyzed esterification of (*R,S*)-*Z*-4-hepten-2-ol with dodecanoic acid [7]. The determination of the configuration of the levorotatory alcohol was performed *via* its (*R*)-2-phenylpropionic acid ester by ^{1}H NMR spectroscopy [12]. Recently, this technique has been successfully applied for the evaluation of absolute configuration of 1-octen-3-ol enantiomers [13]. The ^{1}H NMR spectra of (*R*)-2-phenylpropionic acid esters of (*R,S*)- and (−)-*Z*-4-hepten-2-ol are outlined in Fig. 3. In Table I, the ^{1}H NMR data are summarized. As seen from the data outlined in Table I and Fig. 3 (*cf.* upfield shifted signal of C_7-methyl protons), ^{1}H NMR spectroscopy revealed (*S*)-configuration for the levorotatory antipode, *i.e.* (*S*)(−)-*Z*-4-hepten-2-ol.

Banana volatiles mainly consist of esters, alcohols and carbonyl compounds in a quantitative ratio of approximately 95:4:1, respectively. As main constituents of the ester fraction acetates and butanoates of pentan-2-ol and heptan-2-ol have been described [14, 15]. Recently, these alcohols have been found to occur as optically pure (*S*)(+) enantiomers in their C_2, C_4 and C_6 esters identified in banana [3]. However, as shown in our present study, heptan-2-ol and *Z*-4-hepten-2-ol, as free alcohols, were evaluated to

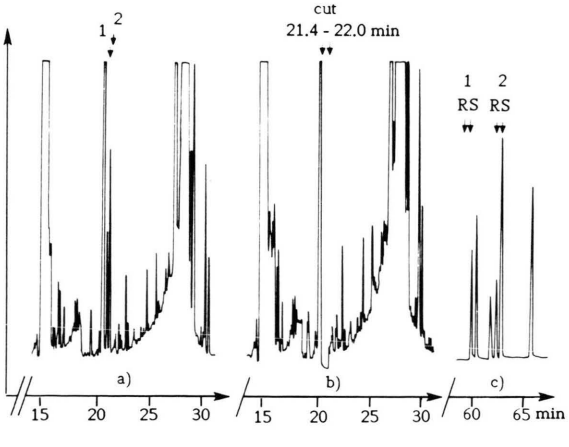

Fig. 2. MDGC separations of IPIC-derivatized fraction II of banana extract; (**1**) heptan-2-ol; (**2**) *Z*-4-hepten-2-ol; a) preseparation on DB-5; b) preseparation on DB-5 with cut; c) separation of diastereomers on XE-60; *cf.* experimental section for details.

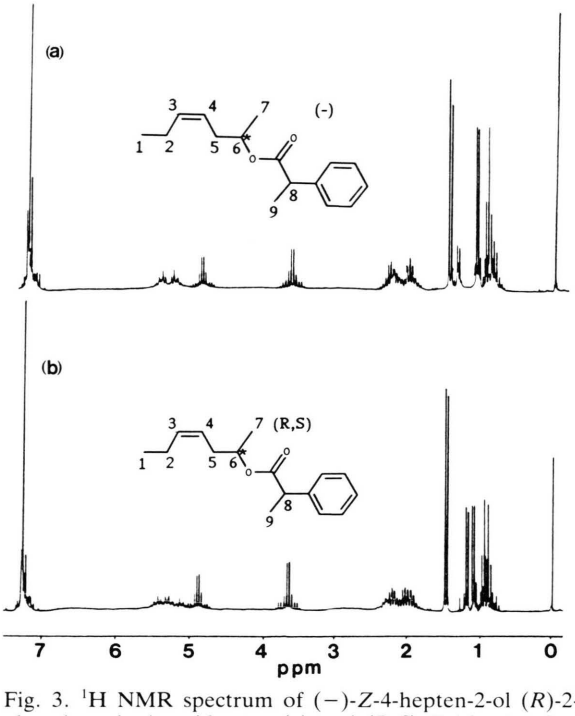

Fig. 3. ^{1}H NMR spectrum of (−)-*Z*-4-hepten-2-ol (*R*)-2-phenylpropionic acid ester (a) and (*R,S*)-*Z*-4-hepten-2-ol (*R*)-2-phenylpropionic acid ester (b); (200 MHz, $CDCl_3$, Me_4Si).

Table I. Chemical shifts of *Z*-4-hepten-2-ol (*R*)-2-phenylpropionic acid esters (ppm, 200 MHz, $CDCl_3$, internal standard Me_4Si).

Alcohol	7[1] (d)	6 (q)	5 (m)	4 (d–t)	3 (d–t)	2 (d–q)	1 (t)
(*R,S*)-	1.17 1.08	4.85	2.23	5.42 5.23	5.26 5.14	1.92	0.95 0.89
(−)-	1.10	4.88	2.30	5.43	5.28	2.00	0.96

[1] Number of the C atom, the protons are bound to, *cf.* Fig. 3.

be present in banana in the above mentioned enantiomeric ratios, but not in optically pure forms.

In the literature, similar results have been previously described for passion fruit, in which different enantiomeric ratios have been determined for alkan-2-ols originating from alcohol and ester fraction [16]. While in the red cultivar (*Passiflora edulis* Sims.) heptan-2-ol has been established to be mainly present as (*R*) enantiomer (92%), in the yellow cultivar (*P. edulis* Sims. forma *flavicarpa* Degener) the (*S*) antipode (82%) predominated. In the ester fraction of the red cultivar, however, optically pure (*R*) enantiomer has been determined. In agreement with our findings these results indicate the activity of at least two different biogenetic pathways giving rise to different enantiomeric ratios of secondary alcohols, as also recently postulated for their biogenesis in corn [17].

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