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Short Communication

Simultaneous stereoanalysis of 2-alkyl-branched acids, esters and alcohols using a selectivity-adjusted column system in multi-dimensional gas chromatography

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ABSTRACT

The direct and simultaneous stereodifferentiation of 2-methylbutanoic acid, 2-methylbutanoic acid methyl and ethyl esters and the corresponding alcohol 2-methylbutane-1-ol from complex matrices is achieved, using selectivity-adjusted multi-dimensional gas chromatography with perethylated β-cyclodextrin as the chiral stationary phase.

INTRODUCTION

Enzymatic reactions are commonly characterized by high stereoselectivity. In this respect, the evaluation of fruit-specific enantiomeric distributions has proved to be an appropriate criterion to differentiate natural flavour compounds from those of synthetic origin. Enantioselective multi-dimensional gas chromatography (MDGC) [1,2] is well established as a technique for the assignment of the origin of γ(δ)-lactones and also many chiral monoterpene compounds of essential oils [3–5].

In many instances, several chiral volatile compounds with different functionalities mainly contribute to the characteristic flavour of fruits and other foodstuffs. Hence chiral stationary phases of versatile enantioselectivity are highly desirable in capillary gas chromatography (cGC). Recently, the first simultaneous stereodifferentiation of all aroma-relevant γ(δ)-lactones from different fruits was described as the latest advance in the analytical differentiation between "natural" and "nature-identical" flavour compounds [6]. Using perethylated β-cyclodextrin as the chiral stationary phase, 2-methylbutanoic acid (**4**), 2-methylbutanoic acid methyl (**1**) and ethyl esters (**2**) and the corresponding alcohol 2-methyl-1-butanol (**3**), some of the most important chiral compounds of apple aroma, were separable into their mirror images [7,8].

This paper describes the direct and simultaneous chirality evaluation of all these substances from complex flavour extracts, using selectivity-adjusted MDGC.

EXPERIMENTAL

Sample preparation

Commercially available apple aroma concentrate A (B) was extracted with pentane–dichloromethane (2:1) under acidic conditions.

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Multi-dimensional gas chromatography

A Siemens SiChromat 2–8 double-oven system equipped with a "live-switching" coupling piece and two flame ionization detectors was used with hydrogen as the carrier gas and with split injection.

Open-tubular columns

Duran glass tubing (Schott Ruhrglas, Mainz, Germany) was drawn into capillaries of 0.23 or 0.32 mm I.D. using a Shimadzu GDM 1 glass-drawing machine. Acid leaching, rinsing and deactivation were performed as described [9–12].

Precolumn system

A 25 m × 0.23 mm I.D. restriction capillary, deactivated with diphenyltetramethyldisilazane (Fluka, Buchs, Switzerland) was coupled with a press-fit connection and a 5-cm fused-silica capillary to a 25 m × 0.32 I.D. glass capillary, deactivated with hexamethyldisilazane, coated with an 18.8% solution of PS-255 (a methylsilicone; Fluka) and 1.5% dicumyl peroxide (Aldrich, Milwaukee, WI, USA) in *n*-pentane–dichloromethane (1:1, v/v).

Main column system I

A 38 × 0.23 mm I.D. glass capillary column, deactivated with phenyldimethyldisilazane (Fluka), was coated with a 0.33% solution of heptakis (2,3,6-tri-O-ethyl)-β-cyclodextrin (33% in OV-1701-vi) in *n*-pentane–dichloromethane (1:1, v/v) [10,11]. The chiral stationary phase was synthesized as described [12].

Main column system II

Non-chiral main column. A 3 m × 0.32 mm I.D. fused-silica capillary coated with a 0.25-μm thick film of DB-1701 (J&W, Carlo Erba, Hofheim, Germany) was used.

Chiral main column. See main column system I.

Heart cutting

The chronological order of the "heart cuts" was as follows, with the cut time adapted to the amounts of the substances: **3**, 14.45–14.55; **1**, 18.15–18.45; **2**, 24.75–25.05; and **4**, 25.80–26.50 min.

RESULTS AND DISCUSSION

The enantiomeric distribution of chiral flavour

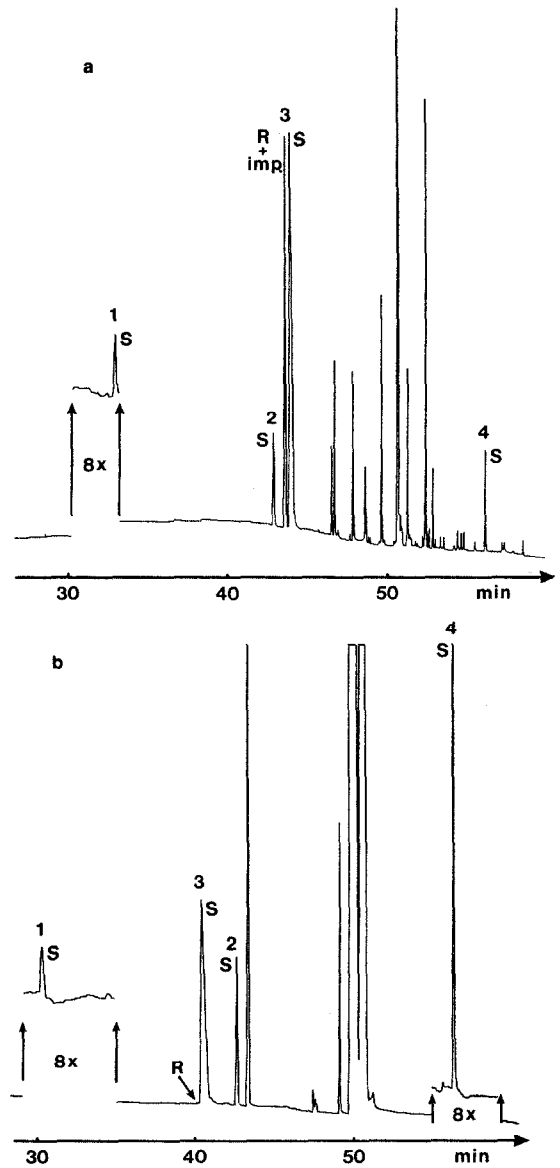

Fig. 1. (a) Enantioselective MDGC analysis of a genuine apple aroma concentrate A (main column system I). Temperature programme of the precolumn: 60°C isothermal for 20 min, then increased at 8°C/min to 240°C. Temperature programme of the main column: 40°C isothermal for 40 min then increased at 2°C/min to 50°C, 10°C/min to 90°C, 1.5°C/min to 105°C and 15°C/min to 210°C. (b) Enantioselective MDGC analysis of the same apple aroma concentrate (main column system II). Temperature programme of the precolumn: 60°C isothermal for 20 min, then increased at 8°C/min to 240°C. Temperature programme of the main column: 40°C isothermal for 35 min, then increased at 2°C/min to 50°C, 15°C/min to 90°C, 1.5°C/min to 110°C and 15°C/min to 210°C.

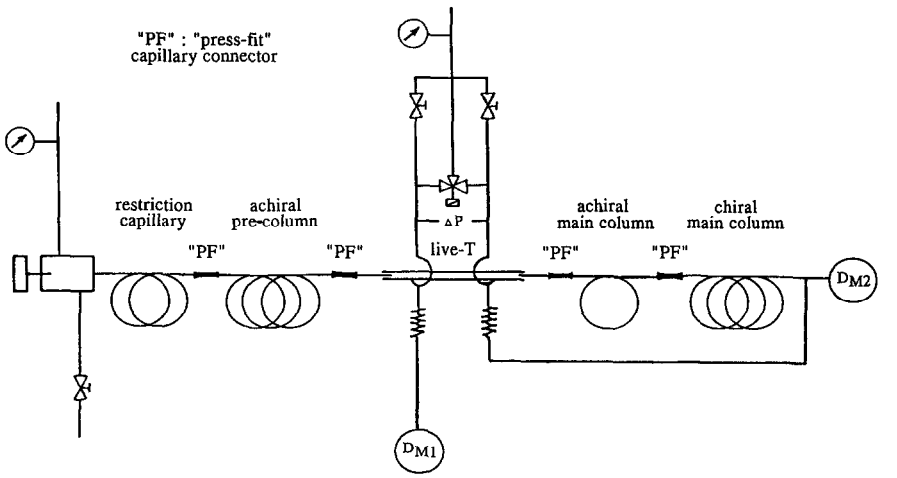

Fig. 2. Column switching in the applied MDGC system.

compounds in natural products reflects the enantiospecificity of their biogenesis. Therefore, the screening of enantiomer composition is a convenient method for differentiating between natural and nature-identical flavour compounds. Compounds **1**–**4** are well known to be some of the most important flavour compounds of apple aroma and their enantioselective analysis has been reported recently using heptakis (2,3,6-tri-O-ethyl)-β-cyclodextrin as a chiral CGC phase [8].

MDGC with a Superox 0.6 non-chiral precolumn and perethylated β-cyclodextrin as the chiral main column was applied previously to investigate the enantiomeric distribution of **1** and **2** [8]. However, this column combination was not suitable for separating the isomeric alcohol 3-methyl-1-butanol (isoamyl alcohol), also a well known component of apple aroma, from the stereoisomers of **3**. Therefore, the column combination had be optimized.

A column coated with a thick film of methylsilicone PS-255 seemed suitable as the precolumn in MDGC for further investigations. The stereoisomeric alcohols were separated at an elution temperature of about 60°C. Using the new column combination, all enantiomers of a standard mixture were baseline resolved. On analysing an apple aroma extract A for the mentioned chiral aroma compounds, the limitations of this configuration were established. Fig 1a shows the chromatogram obtained. The esters **1** and **2** and the acid **4** are detected as pure *S*-enantiomers. The enantiomer distribution of **3** seems to be different. A "single cut" of the alcohol indicated that this would be a misinterpretation; in fact, **3** has a nearly pure *S*-configuration. This means that unknown substances being transferred from the precolumn co-elute with **3** after their passage through the chiral main column. Obviously, the selectivities of the pre- and the main columns were too different.

Different columns with various polarities and columns coated with thick films of mixed stationary phases were tested to overcome this problem, without success.

An alternative approach was to adapt the pre- and the main columns by the additional insertion of a short non-chiral main column, coupled directly to the chiral main column with a press-fit connection (Fig. 2). A test with a 3 m × 0.32 mm I.D. fused-silica capillary coated with Supelcowax 10M (film thickness 0.25 μm) was not successful. The elution temperature of **4** was too high for the stereodifferentiation on perethylated β-cyclodextrin as the chiral stationary phase. Subsequently a 3 m × 0.32 mm I.D. fused-silica capillary coated with DB-1701 (film thickness 0.25 μm) was used. This combination was expected to be useful as the chiral material was dissolved in OV-1701-vi also.

The insertion of this short column had a very significant effect on the resulting chromatogram. The elution order of **3** and **2** is changed (Fig. 3). The

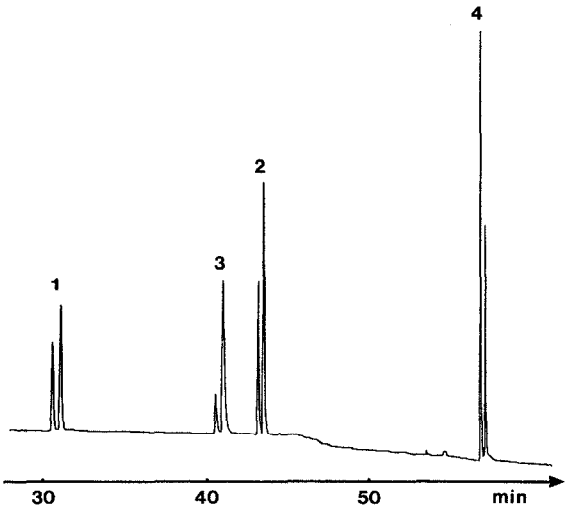

Fig. 3. Simultaneous enantioselective MDGC analysis of a standard mixture of methyl 2-methylbutanoate (**1**), ethyl 2-methylbutanoate (**2**), 2-methyl-1-butanol (**3**) and 2-methylbutanoic acid (**4**), all compounds *S*-enriched (main column system II). Temperature programme as in Fig. 1b.

resolution of the enantiomers is not affected by this added non-chiral main column.

Repeated analysis of the same apple aroma extract showed an improved accuracy. The enantiomer distribution of all the investigated compounds is now exactly determined (Fig. 1b).

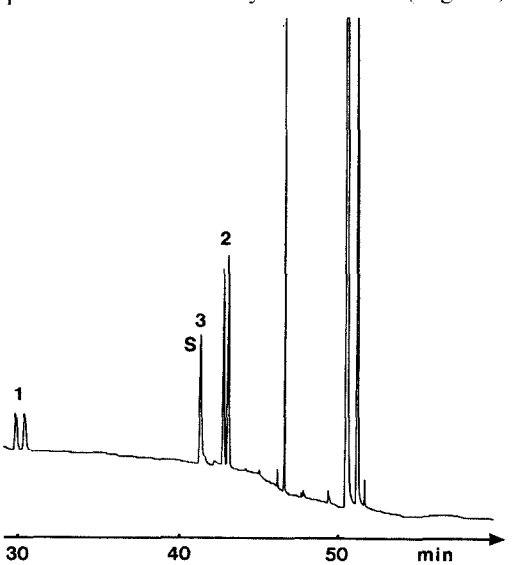

Fig. 4. Enantioselective MDGC analysis of an apple aroma concentrate B, adulterated by synthetic racemates of **1** and **2** (main column system I). Temperature programme as in Fig. 1b.

The analysis of another apple aroma concentrate (B) showed the improved speed of differentiation between natural and nature-identical compounds **1**–**3** (Fig. 4). After extraction with *n*-pentane–dichloromethane (2:1), the addition of nature-identical **1** and **2** was clearly detected by only one MDGC analysis.

CONCLUSIONS

Enantioselective inclusion GC with heptakis (2,3,6-tri-O-ethyl)-β-cyclodextrin as the chiral phase is an effective method for differentiating the mirror images of 2-methylbutanoic acid (**4**), the esters **1** and **2** and the corresponding alcohol **3**.

MDGC employing the heart-cutting technique from a suitable non-chiral precolumn onto a chiral main column, coated with perethylated β-cyclodextrin as the chiral stationary phase, allows the direct and simultaneous evaluation of the chirality of **1**–**4** from complex flavour matrices when an additional short nonchiral main column is inserted. The described modification of the main column system proved to be an efficient approach to realizing the simultaneous evaluation of the chirality of flavour compounds with different functionalities.

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