Stereoisomeric Flavor Compounds. 72. Stereoisomeric Distribution of Some Chiral Sulfur-Containing Trace Components of Yellow Passion Fruits

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The enantiomeric distributions of *cis*- and *trans*-2-methyl-4-propyl-1,3-oxathiane and 3-mercaptohexyl acetate and butanoate in passion fruit extracts were determined by means of multidimensional gas chromatography (MDGC) using sulfur-selective detection. 4*S*-configured oxathianes and *S*-configured 3-mercaptohexyl alkanoates of high enantiomeric purity were detected. 2,2-Dimethyl-4-propyl-1,3-oxathiane and 3-mercaptohexyl propanoate were used as internal standard components, allowing a quantification of the volatiles investigated. The trace components were found in a range from less than 0.5 to 18 ppb. The results are discussed with regard to the biosynthesis of these volatiles. Due to the high enantiomeric purities detected, the differentiation between naturally occurring flavor compounds and synthetic racemates added to passion fruit products is realized conclusively.

Keywords: *Enantioselective GC analysis; multidimensional gas chromatography (MDGC); sulfur-selective detection; chiral sulfur-containing flavor compounds; yellow passion fruit*

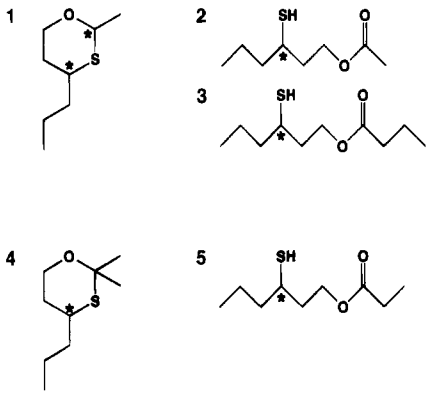
INTRODUCTION

The chiral sulfur-containing flavor compounds 2-methyl-4-propyl-1,3-oxathiane (**1**), 3-mercaptohexanol, 3-(methylthio)hexanol and their acetates, butanoates, and hexanoates are among the most potent components responsible for the typical tropical-fruity notes of the yellow passion fruit (Winter et al., 1976; Engel and Tressl, 1991). All stereoisomers of these chiral sulfur-containing volatiles have been synthesized with high enantiomeric purity. Chirality evaluation of compound **1** and enantiomeric ratios of the above-mentioned genuine alcohols from the yellow passion fruit have been described using enantioselective gas chromatography (Heusinger and Mosandl, 1984; Mosandl and Heusinger, 1984, 1985; Heusinger, 1986; Singer et al., 1986; Mosandl, 1992; Weber et al., 1992, 1994).

Due to their importance to the flavor of the yellow passion fruit, the detection of adulterations of passion fruit products by the addition of synthetic sulfur-containing flavor compounds is of considerable interest. Therefore, the evaluation of the origin-specific enantiomeric distribution of the stereoisomers for differentiating the natural volatiles from those of synthetic origin is imperative. As enantiomers of chiral sulfur-containing volatiles often show clear differences in their sensorial properties, the stereodifferentiation of the naturally occurring sulfur-containing flavor compounds should give a better insight into their role as passion fruit flavor compounds.

This paper reports on the determination of the stereoisomeric distributions of 2-methyl-4-propyl-1,3-oxathiane (**1**) and 3-mercaptohexyl acetate (**2**) and butanoate (**3**) (Chart 1) in passion fruit extracts by means of multidimensional gas chromatography (MDGC) using sulfur-selective detection. The new cyclodextrin derivative octakis(2,3-di-*O*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (dibutyryl-6-TBDMS-γ-CD) was used as the chiral stationary phase of the main column of the MDGC system (Schmarr, 1992). 2,2-Dimethyl-4-propyl-1,3-oxathiane (**4**) and 3-mercaptohexyl propanoate (**5**) were used as internal standard components, allowing quantification of the volatiles investigated.

Chart 1



MATERIALS AND METHODS

Materials. Yellow passion fruits (1592 g) and 2.1 L of passion fruit nectar (fruit content 25%) were purchased at a local market. One kilogram of passion fruit juice concentrate was supplied by a commercial flavor company.

Sample Preparation. The fruits were cut into two pieces, and the pulp was separated by pressing through a sieve. (*S*)-**4** (6 μg) and (*S*)-**5** (7 μg) were added, and the pulp was subsequently filled into a 5 L liquid–liquid extractor (Ludwig, 1972), topped up to 5 L with deionized water and extracted with pentane/dichloromethane (2 + 1, v + v) within 14 h. The aroma extract was dried over Na_2SO_4 and concentrated to a volume of approximately 100 mL. To remove components, which may deteriorate the efficiency of the gas chromatographic column (Weber, 1995), the extract was purified by a silica gel column (1 g, Merck 7734 with 10% H_2O) in batches of 25 mL. Each silica gel column was treated with a further 10 mL of pentane/dichloromethane (2 + 1, v +

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v). The fractions were combined and concentrated to a volume of approximately 0.3 mL, using a Vigreux column. The same procedure was carried out with 2.1 L of passion fruit nectar and 200 g of passion fruit juice concentrate, respectively.

Capillary Gas Chromatography. A DB-210 fused silica column (30 m × 0.32 mm i.d., film thickness 0.25 μm) was used as the precolumn and a fused silica column coated with 50% octakis(2,3-di-*O*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin in OV-1701-vi (30 m × 0.32 mm i.d., film thickness 0.32 μm) as the main column. Both columns were installed into a Fisons GC 8000 gas chromatograph and coupled via a moving capillary stream switching (MCSS) system (Fisons Instruments, Mainz-Kastel, Germany). Detectors were a flame ionization detector on the MCSS system outlet and a flame photometric detector on the main column outlet. A split/splitless injector was used, and injection volumes were 1 μL (90 s splitless). The injector temperature was 220 °C, and the detector temperatures were 240 °C. The data recording as well as the control of the MCSS system was managed with a personal computer and Chrom-Card for Windows software (Fisons Instruments). The carrier gas was helium at 1.0 mL/min (precolumn) and 1.3 mL/min (main column), respectively. The temperature program was 5 min isothermal at 40 °C, 40–105 °C at 5 °C/min, 22 min isothermal at 105 °C, 105–150 °C at 1.5 °C/min, 150–220 °C at 5 °C/min, and finally 30 min isothermal at 220 °C for the investigation of the passion fruit nectar and the concentrate. The retention times on the precolumn were as follows: *cis*-**1**, 18.73 min; **4**, 19.82 min; *trans*-**1**, 20.28 min; **2**, 29.51 min; **5**, 35.40 min; and **3**, 44.64 min. Transfers onto the main column using cut time intervals were as follows: *cis*-**1**, **4**, and *trans*-**1**, 18.20–20.80 min; **2**, 29.00–30.20 min; **5**, 34.90–36.10 min; and **3**, 44.10–45.60 min. The precolumn was backflushed after 48.00 min. The retention times on the main column were as follows: *cis*-(2*R*,4*S*)-**1**, 33.74 min; (*S*)-**4**, 37.08 min; (*R*)-**4**, 38.90 min; *cis*-(2*S*,4*R*)-**1**, 39.41 min; *trans*-(2*R*,4*R*)-**1**, 41.52 min; *trans*-(2*S*,4*S*)-**1**, 46.75 min; (*R*)-**2**, 51.72 min; (*S*)-**2**, 52.38 min; (*R*)-**5**, 59.67 min; (*S*)-**5**, 60.11 min; (*R*)-**3**, 67.26 min; and (*S*)-**3**, 67.49 min. Because of deterioration of the precolumn efficiency, the investigation of the yellow passion fruit aroma extract had to be carried out with the following temperature program: 5 min isothermal at 40 °C, 40–100 °C at 5 °C/min, 30 min isothermal at 100 °C, 100–150 °C at 1.5 °C/min, 150–220 °C at 5 °C/min, and finally 30 min isothermal at 220 °C. The retention times on the precolumn were as follows: *cis*-**1**, 18.85 min; **4**, 20.04 min; *trans*-**1**, 20.58 min; **2**, 31.68 min; **5**, 39.09 min; and **3**, 51.21 min. In a first investigation with this temperature program *cis*- and *trans*-**1** could not be detected, but the (*R*)-**2** stereoisomer coeluted with an unknown sulfur-containing compound, which had been transferred onto the main column during the first "cut". Therefore, the investigations of **2**, **5**, and **3** were carried out without transfer of **1** and **4** onto the main column. The cut time intervals were as follows: **2**, 31.40–32.20 min; **5**, 38.59–39.75 min; and **3**, 50.70–51.70 min. The precolumn was backflushed after 55.00 min. The retention times on the main column were as follows: (*R*)-**2**, 59.81 min; (*S*)-**2**, 60.72 min; (*R*)-**5**, 68.62 min; (*S*)-**5**, 69.17 min; (*R*)-**3**, 76.63 min; and (*S*)-**3**, 76.90 min.

Quantification. For quantitative analysis the obtained peak areas were corrected by working out their square roots (Weber, 1995). The amounts of **1**, **2**, and **3** were calculated by comparison of their corrected peak areas with those of the internal standards **4** and **5** (a response factor of 1 was supposed), respectively.

RESULTS AND DISCUSSION

The aroma extract of the passion fruit nectar was obtained by liquid–liquid extraction (Ludwig, 1972). As the investigated sulfur-containing volatiles **1**–**3** occur in passion fruits in very low concentrations, effective extraction and separation techniques become necessary. The use of **4** and **5** as internal standards allows an efficient control of the sample preparation method applied, as the chemical and physical properties of compounds **4** and **5** are rather similar to those of compounds **1**–**3**. Furthermore, the addition of internal standards enables the quantification of **1**–**3**. As sulfur-containing flavor compounds are known to exhibit different sensorial properties at different concentrations, a quantification of **1**–**3** gives further information for their sensorial meaning to passion fruits.

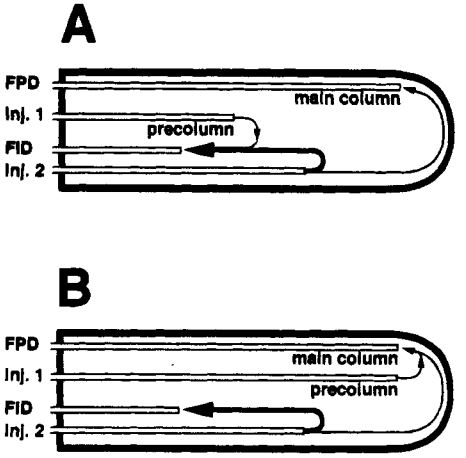

Figure 1. MCSS column coupling system (Fisons Instruments): (A) normal adjustment; (B) adjustment for transfer of precolumn effluents onto the main column.

Stereodifferentiation of **1**–**3** was performed by enantio-MDGC with sulfur-selective detection. Enantio-MDGC has been well proved in quality assurance and origin control of flavors (Mosandl, 1992; Werkhoff et al., 1993, and literature cited therein). To avoid coelutions on the main column, in many cases only a part of the interesting component is transferred from the precolumn onto the main column. Nevertheless, the low amounts of **1**–**3** as well as the quantification method using the internal standard components **4** and **5** required a complete transfer of **1**–**5** onto the main column. This became possible by use of a flame photometric detector revealing only the sulfur-containing volatiles. It should be mentioned that for the determination of enantiomeric distribution, as well as for quantifications, the peak areas obtained had to be corrected by working out their square roots due to the quadratic response of the sulfur-selective detector. Furthermore, a complete transfer by the system applied for coupling the precolumn and the main column had to be secured. A schematic drawing of the MDGC coupling system used, the so-called MCSS system, is shown in Figure 1.

The MCSS system consists of a glass cap tube in which four capillaries are connected in defined distances to each other. The four capillaries are the precolumn, the main column, and two further capillaries; one of the latter capillaries is connected with a second injector, and the other one is connected with a flame ionization detector. The carrier gas reaching the MCSS system via the second injector is divided into the carrier gas stream of the main column (1–2 mL/min) and a stream to the FID (>10 mL/min). The latter takes along the precolumn effluent to the FID as can be seen in Figure 1A. The transfer of the precolumn effluents onto the main column is obtained by moving the precolumn close to the main column (Figure 1B). Transfer rates of 97% were obtained by optimizing the flow adjustments. Thus, the MCSS system is a suitable MDGC coupling system in the analysis of trace components (Weber, 1995).

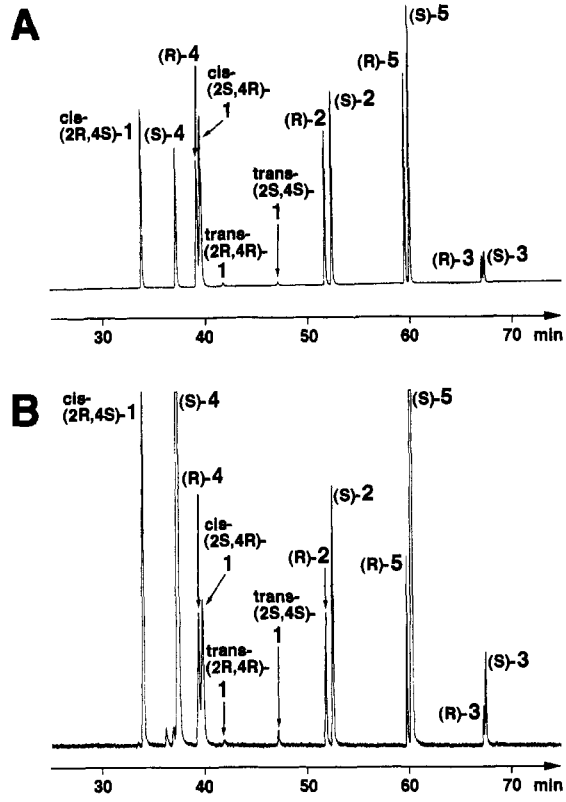

Figure 2. Separation of the stereoisomers of **1**–**5** using octakis(2,3-di-*O*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin as the chiral stationary phase: (A) standard solution (racemates); (b) mixture of the aroma extract of a passion fruit nectar and the standard solution (coinjection).

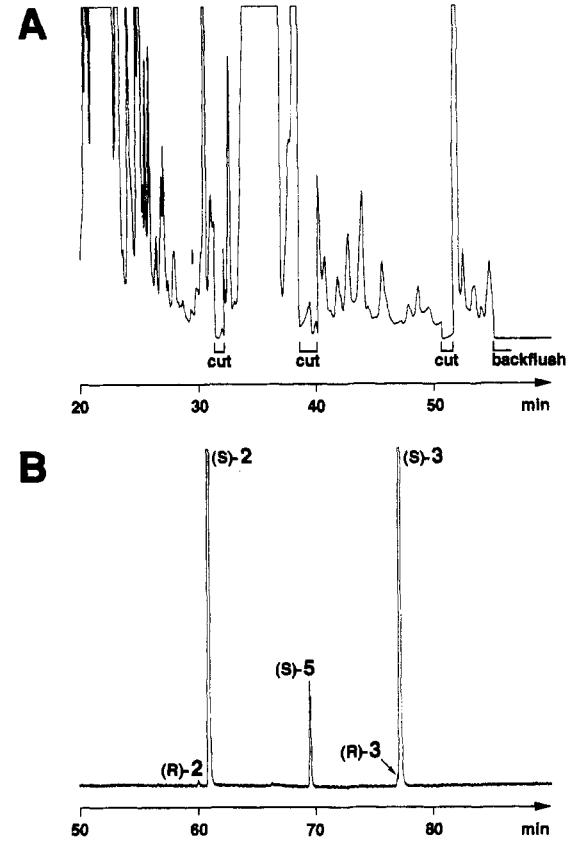

Figure 3. Precolumn (A) and main column (B) chromatogram of the aroma extract of the yellow passion fruit sample.

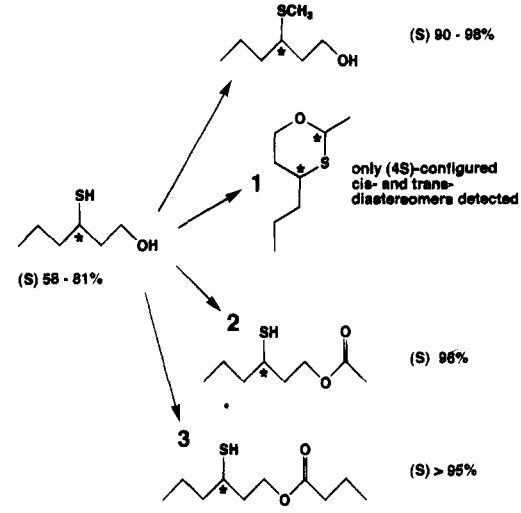

Figure 4. Summary of the stereoisomeric distribution of the sulfur-containing volatiles of the yellow passion fruit with regard to their supposed biosynthetic pathway.

Octakis(2,3-di-*O*-butyryl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (dibutyryl-6-TBDMS-γ-CD) was used as the chiral stationary phase. The synthesis of this cyclodextrin derivative has been described (Schmarr, 1992), but until now no application has been reported. Figure 2a shows the separation of the stereoisomers of **1**–**5** on this stationary phase. The extreme resolutions of the stereoisomers of **1** are noteworthy. Because of the partial coelution of (*R*)-**4** and (2*S*,4*R*)-**1**, as well as for easier quantification, the enantiopure *S*-configured internal standard components **4** and **5** were added prior to sample preparation. Therefore, (*S*)-**4** and (*S*)-**5** predominate over the *R*-configured enantiomers in Figure 2b, detecting the coinjection of the racemic standard solution (Figure 2A) with the genuine aroma extract of a passion fruit nectar. The enrichment of (*S*)-**2**, (*S*)-**3**, and the 4*S*-configured *cis*-**1** and *trans*-**1** becomes obvious, too. Without addition of the standard solution the corresponding *R*-configured stereoisomers could not be detected. The quantification yields **1** ppb for *cis*-(2*R*,4*S*)-**1** and trace amounts (<0.5 ppb) in the case of *trans*-(2*S*,4*S*)-**1**, (*S*)-**2**, and (*S*)-**3**.

The investigation of the yellow passion fruit sample (precolumn chromatogram in Figure 3A) yields comparatively high amounts of (*S*)-**2** (14 ppb) and (*S*)-**3** (18 ppb), as can be seen in the main column chromatogram (Figure 3B). The percentages of *R*-enantiomers are 4% for (*R*)-**2** and estimated less than 5% in the case of (*R*)-**3**, respectively. Stereoisomers of **1** were not detected in this aroma extract. The investigation of a concentrate of yellow passion fruit juice confirmed the results of the other samples investigated, as it shows only *S*-configured compound **3** in a concentration of 10 ppb. Figure 4 summarizes the results obtained for compounds **1**–**3** as well as the known enantiomeric distributions of 3-mercapto- and 3-(methylthio)hexanol (Weber et al., 1994) in connection with their supposed biosynthesis (Tressl and Albrecht, 1986; Tressl et al., 1988). 3-(Methylthio)hexanol, as well as the components **1**–**3**, occurred in yellow passion fruits with high enantiomeric purities in favor of the 3*S*- and 4*S*-configured enantiomers, respectively. 3-Mercaptohexanol, which is considered to be the precursor of the other volatiles, shows also an enrichment of the *S*-enantiomer, but its enantiomeric ratio varies in a wide range from 58 to 81%. A possible explanation is that 3-mercaptohexanol originates at first in high enantiomeric purity in favor of the *S*-enantiomer, but this enantiomer is subsequently used for the highly stereoselective genera-

tion of 3-(methylthio)hexanol and compounds **1**–**3**. Consequently, the actual enantiomeric purity of the remaining of 3-mercaptohexanol is decreasing after all (58–81% of the *S* enantiomer). Further studies confirming this hypothesis should be focused to the stereoisomeric distributions as well as to the quantifications of all chiral sulfur-containing volatiles.

The concentrations of the sulfur-containing flavor compounds obtained are in accordance with reported data, and differences in concentrations between various samples have been described, too (Engel and Tressl, 1991). These differences have to be discussed with regard to the ripeness of the fruits and, in the case of passion fruit products, as a result of processing. Nevertheless, the stereoisomeric distributions of the sulfur-containing volatiles are not influenced by the processing conditions applied. Apart from 3-mercaptohexanol, the high enantiomeric purities, obtained for all investigated chiral sulfur-containing volatiles, conclusively allow differentiation between the naturally occurring flavor compounds and synthetic racemates added to passion fruit products.

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