Stereoisomeric Flavor Compounds. 48. Chirospecific Analysis of Natural Flavors and Essential Oils Using Multidimensional Gas Chromatography

Armin Mosandl,* Katja Fischer, Uwe Hener, Peter Kreis, Klaus Rettinger, Vera Schubert, and Hans-Georg Schmarr

Institut für Lebensmittelchemie, Universität Frankfurt, D-6000 Frankfurt/Main, Germany

Multidimensional gas chromatography (MDGC), employing heart-cutting techniques from a polar and nonchiral preseparation column onto a chiral main column, coated with heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin as the chiral stationary phase, is proved to be a rather sensitive method of high selectivity to differentiate the enantiomers of 2-methylbutanoic acid (esters), chiral monoterpenes, alkan-2-ols, alken-2-ols, and 1,2-ketols from complex matrices. The specific distribution of enantiomers is discussed as an indicator for the genuineness of natural flavors and essential oils.

INTRODUCTION

Very recently, capillary gas chromatography (CGC), using modified cyclodextrins as chiral stationary phases, has proved to be a powerful tool for the enantiodifferentiation of chiral volatiles (Schurig and Nowotny, 1988; Nowotny et al., 1989; König et al., 1988, 1989a, 1990; Mosandl et al., 1990a; Takeoka et al., 1990). This is of considerable interest with respect to the fruit specific distribution of enantiomers in nature and to their importance for the genuineness of natural flavors and essential oils. By means of multidimensional gas chromatography (MDGC) (Chinghai et al., 1983; Schomburg et al., 1984; Nitz et al., 1989), employing heart-cutting techniques from a polar (nonchiral) preseparation column onto a chiral main column, coated with modified β-cyclodextrin chiral γ-lactones were directly stereoanalyzed from complex matrices (Mosandl et al., 1989). This paper reports on the enantiomeric distribution of 2-methylbutanoic acid in apples (Rettinger et al., 1990; Rettinger, 1991) and on the stereodifferentiation of chiral alcohols as acetates from bananas (Schubert, 1991).

The differentiation of the chiral monoterpenes α-pinene, β-pinene, and limonene from essential oils and the evaluation of chiral 1,2-ketols from honey are also reported (Fischer and Mosandl, 1990).

EXPERIMENTAL PROCEDURES

Optically Pure References. The structure and properties of optically pure references are reported in previous papers: 2-methylbutanoic acid (esters) (Rettinger, 1990); pentan-2-ol (**1**), hexan-2-ol (**2**), heptan-2-ol (**3**) enantiomers (Deger, 1987); (*Z*)-4-hepten-2-ol (**4**) (Schubert, 1991); 3-hydroxybutan-2-one (**8**), 2-hydroxypentan-3-one (**9**), 3-hydroxypentan-2-one (**10**) (Fischer, 1990; Fischer and Mosandl, 1990). The optically active monoterpenes α-pinene (**5**), β-pinene (**6**), and limonene (**7**) are commercially available (Aldrich).

Instrumental Setup. Analyses were performed with a Siemens SiChromat 2 multidimensional gas chromatography system, containing two ovens with independent temperature programs, equipped with two flame ionization detectors (FID) and the "live-switching" coupling piece. Injection mode: split 1:25. Injection temperature: 210 °C. Detection temperature: 230 °C each.

Separation Conditions: Carrier gas, H_2; preseparation pressure, P_A; main separation pressure, P_M. Figures 1 and 2: P_A = 1.75 bar, P_M = 1.30 bar. Figures 3–7: P_A = 1.25 bar, P_M = 1.05 bar.

Preseparation. A chemically bonded poly(ethylene glycol) fused silica column (Supelcowax 10), 60 m × 0.32 mm (i.d.) with 0.25-μm film thickness was used. For programs, cf. Figures 1–7.

Main Separation. A Duran glass capillary column, 47 m × 0.23 mm (i.d.), was leached and deactivated with diphenyltetramethyldisilazane (DPTMDS, Fluka) and statically coated with a 10% solution of heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin in OV-1701-vi to form a film of 0.3-μm thickness (Schurig and Nowotny, 1988). For programs, cf. Figures 1–7.

Sample Preparation. (*1*) *2-Methylbutanoic Acid from Apples.* Four apples (0.8 kg, Granny Smith) are washed, peeled, homogenized, and squeezed. For enzyme inhibition the eluting apple juice is immediately mixed with 150 g of $(NH_4)_2SO_4$ (Guichard and Souty, 1988). The mixture is extracted with three portions of pentane/dichloromethane (2:1 v/v), 200 mL each, the emulsion is filtered through an emulsion-breaking filter, and the resulting organic layer is dried with $MgSO_4$, filtered, and concentrated to about 50 mL with a Vigreux column. The organic layer is washed five times with 25 mL of $NaHCO_3$ solution (5% in H_2O) to cause hydrolysis of 2-methylbutanoic acid esters (Idstein et al., 1985).

Workup conditions: The organic solution is removed and the aqueous layer adjusted to pH 2 with 6 N H_2SO_4. After saturation with NaCl, free 2-methylbutanoic acid is extracted with three portions of dichloromethane, 50 mL each, collected, dried with $MgSO_4$, and concentrated to about 0.5 mL with a Vigreux column. The solution is ready to use for MDGC.

(*2*) *Chiral Alcohols from Bananas.* Ripe bananas (650 g) from Honduras are peeled and homogenized with 650 g of $(NH_4)_2SO_4$ and 100 mL of water and extracted with 300 mL of pentane/diethyl ether (1:1 v/v) over 3 days (20 °C). Subsequently, the organic layer is separated, dried with Na_2SO_4, and concentrated to about 1.0 mL with a Vigreux column.

Isolation and acetylation of the alcoholic fraction: One part of the concentrated extract is separated by preparative thin-layer chromatography, using silica gel Si60 (2 mm) as the sorbent and pentane/diethyl ether (9:1 v/v) as the eluent.

The alcoholic fraction [R_f 0.14; reference, (*Z*)-4-hepten-2-ol] is removed from the silica gel plate and the sorbent extracted with 4 mL of pentane/diethyl ether (1:1 v/v). The chiral alcohols are esterified to their acetates by using 200 μL of acetyl chloride and 4-(dimethylamino)pyridine as a catalyst and stirred overnight (20 °C).

Workup conditions: The solution is washed with 10 mL of 2 N HCl, saturated solutions of $NaHCO_3$ (10 mL), and NaCl (10 mL). After drying over Na_2SO_4 and concentrating to about 1.0 mL the solution is ready for analysis by MDGC.

(*3*) *Essential Oils.* Commercially available essential oils are simply diluted to 0.5% with *n*-pentane for analysis by MDGC. Injection volumes: 0.3–1.0 μL, depending on the concentration of chiral volatiles to be analyzed.

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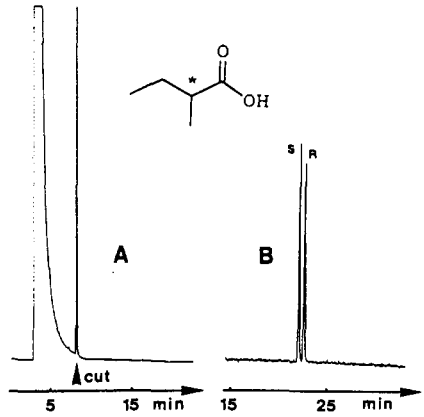

Figure 1. (A) Preseparation of nature-identical (racemic) 2-methylbutanoic acid (after ester hydrolysis) on Supelcowax TM 10. Program: 140 °C/10 min isothermal, 2 °C/min → 160 °C/10 min isothermal, 3 °C/min → 225 °C/30 min isothermal. (B) Transfer of nature-identical (racemic) 2-methylbutanoic acid onto the main column and base-line resolution with permethylated β-cyclodextrin as the chiral stationary phase. Program: 105 °C isothermal. Order of elution by co-injection with optically pure references (Mosandl et al., 1990; Rettinger et al., 1990).

(4) Chiral 1,2-Ketols from Honey. Honey (0.5 kg) is dissolved with water (1000 mL) in a three-necked flask. The solution is heated, stirred, and steam distilled; 200 mL of the distillate is saturated with NaCl and exhaustively extracted with dichloromethane. The organic layer is dried with Na_2SO_4, filtered, and concentrated to about 1.0 mL with a Vigreux column. This solution is ready for injection to MDGC.

Note: The described procedure occurs *without any racemization*, as proved by model experiments with chiral ketols of definite enantiomeric ratios (Fischer, 1990).

cut no.	transfer interval, min	transferred 1,2-ketol
1	17.8–18.2	8
2	22.0–22.4	10
3	23.6–24.0	9

RESULTS AND DISCUSSION

Multidimensional gas chromatography (MDGC) (Chinghai et al., 1983; Schomburg et al., 1984; Nitz et al., 1989), employing heart-cutting techniques from DB-1701 onto heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin as the chiral main column, has proved to be an efficient and rather sensitive method for the direct chiral resolution of γ-lactones from complex matrices without any further cleanup procedure (Mosandl et al., 1989).

Permethylated β-cyclodextrin has been recently reported as a versatile chiral stationary phase for the enantiospecific CGC analysis of chiral flavor compounds with different functionalities (Mosandl et al., 1990a). Using Carbowax 20M as a nonchiral precolumn and transfer onto permethylated β-cyclodextrin as the chiral main column, a successful application of the MDGC technique to the chirality evaluation of 2-methylbutanoic acid (esters) from Granny Smith apples is achieved.

As outlined in Figure 1 the differentiation of enantiomers of 2-methylbutanoic acid and their esters (after hydrolysis) from apples and other complex flavor essences is achieved without any racemization (Rettinger et al., 1990). While "nature-identical" ethyl 2-methylbutanoate is detected as a racemic mixture (Figure 1), the natural flavor impact compound from Granny Smith apples is of high optical purity in favor of the *S* configuration (Figure 2).

The first reports on the composition of banana flavor compounds revealed pentan-2-ol, heptan-2-ol, and (*Z*)-4-hepten-2-ol and their acetates (esters) as characteristic aroma compounds (Tressl et al., 1969).

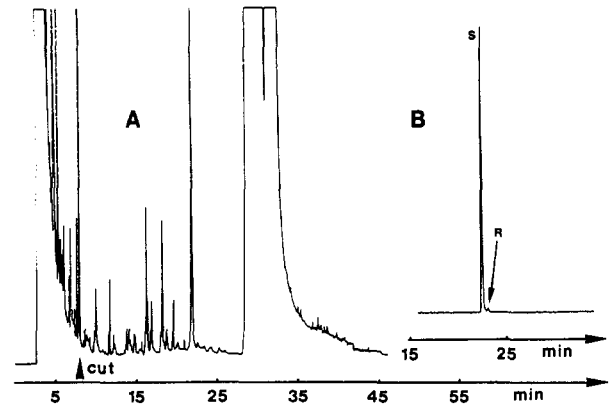

Figure 2. (A) Hydrolyzed raw apple flavor extract, preseparated with Supelcowax TM 10. Cut: eluate transfer onto the chiral main column. Conditions: see Figure 1A. (B) Chirality evaluation of natural 2-methylbutanoic acid (after ester hydrolysis) from Granny Smith apples, transferred from Supelcowax TM 10 onto permethylated β-cyclodextrin. Conditions: see Figure 1B.

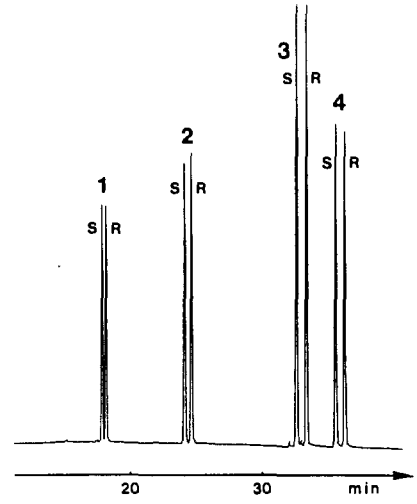

Figure 3. Standard mixture and base-line resolution of racemic acetates of pentan-2-ol (1), hexan-2-ol (2), heptan-2-ol (3), and (*Z*)-4-hepten-2-ol (4) using permethylated β-cyclodextrin as the chiral stationary phase. MDGC conditions: see Figure 1. Preseparation program: 70 °C/10 min isothermal, 3 °C/min → 200 °C. Main separation program: 95 °C/10 min isothermal, 0.5 °C/min → 150 °C. Order of elution assigned with optically pure references (Deger, 1988; Mosandl and Deger, 1989; Schubert, 1990).

By stereodifferentiation via derivatization diastereomeric *S*-configured alkan-2-yl esters (Gessner et al., 1988) and chiral carbamates of moderate *S* enantiomeric excess have been detected (Fröhlich et al., 1989).

This investigation deals with detailed results on the enantiomeric distribution of chiral alcohols from bananas after preseparation by microscale thin-layer chromatography, subsequent acetylation, and MDGC.

Chiral alcohols at high optical purity in favor of the *S* configuration are detected: pentan-2-ol (96.6% *S*); hexan-2-ol (98.1% *S*); heptan-2-ol (83.8% *S*); (*Z*)-4-hepten-2-ol (95.6% *S*) (Figures 3 and 4).

In previous studies the enantiomeric distribution of α-pinene (5), β-pinene (6), and limonene (7) from essential oils, from extracts and products of the pharmaceutical and perfume and food industries, has been analyzed (Mosandl et al., 1990b; Hener et al., 1991a,b; Kreis et al., 1991). This paper extends our investigations into the stereodifferentiation of 5–7 from mint oils (Table I). As outlined in Table II, mint oils of Brazilian and Japanese types are

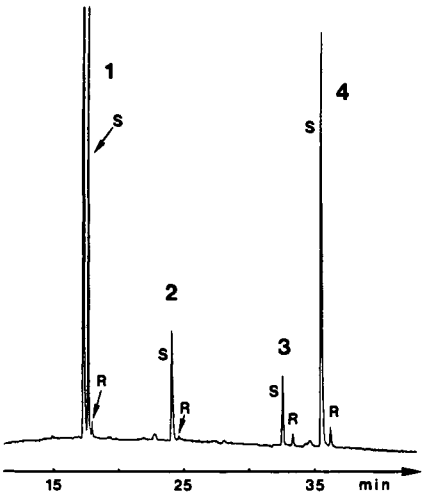

Figure 4. Stereodifferentiation of the acetylated alcohol fraction from the flavor extract of ripe bananas. MDGC conditions: see Figure 3.

Table I. Mint Oils Investigated (Commercial Samples)

common oil name	systematic name
Mentha citrata	*Mentha aquatica* L. × *Mentha viridis* L.
mint, Brazil	*Mentha arvensis* L. var. Piperascens
mint, Japan	*Mentha arvensis* L. var. Piperascens
peppermint	*Mentha piperita* L.
spearmint	*Mentha spicata* L.

Table II. Enantiomeric Ratio (%) of α-Pinene (5), β-Pinene (6), and Limonene (7) in the Investigated Mint Oils [Accuracy: 5 (±0.5%); 6 (±0.5%); 7 (±1.0%)]

	α-pinene		β-pinene		limonene	
common oil name	*S*	*R*	*S*	*R*	*S*	*R*
Mentha citrata	21	79	92	8	8	92
mint, Brazil	73	27	53	47	99	1
mint, Japan	72	28	53	47	100	tr
peppermint 1	70	30	54	46	98	2
peppermint 2	66	34	52	48	98	2
spearmint	43	57	60	40	81	19

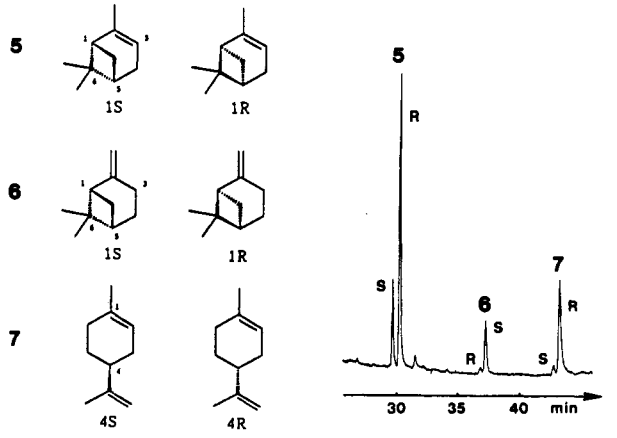

Figure 5. Enantiodifferentiation of α-pinene (5), β-pinene (6), and limonene (7) from the essential oil of *Mentha citrata*, using MDGC and the column combination as outlined in Figure 1. Preseparation program: 70 °C/20.5 min isothermal, 20 °C/min → 90 °C. Main separation program: 60 °C/20.5 min isothermal, 20 °C/min → 80 °C.

nearly identical and peppermint oils from different origins are found to be in accordance with each other. Nevertheless, all investigated *Mentha* species are differentiated with regard to their enantiomeric distribution of 5–7 (Table II; Figure 5).

Chiral 1,2-ketoalcohols are known to be characteristic neutral volatiles of many fermented food and beverages,

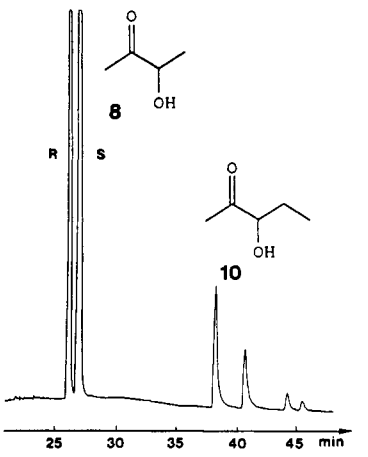

Figure 6. Chirospecific analysis of 3-hydroxybutan-2-one (8) and 3-hydroxypentan-2-one (10) from the steam distillate of honey, using MDGC conditions as outlined in Figure 1. Preseparation program: 100 °C isothermal. Main separation program: 65 °C isothermal. Transfer conditions: see Sample Preparation.

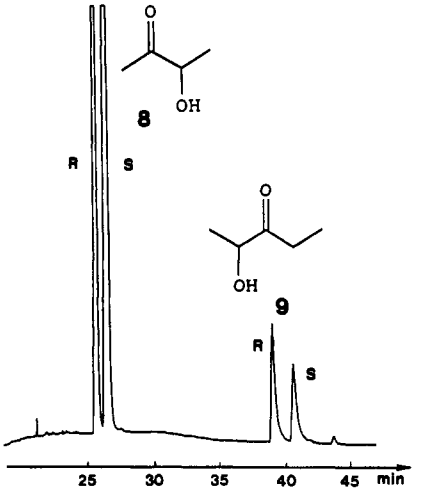

Figure 7. Chirospecific analysis of 3-hydroxybutan-2-one (8) and 2-hydroxypentan-3-one (9) from the steam distillate of honey, using MDGC conditions as outlined in Figure 1. Programs: see Figure 6.

indicating an intense reductive activity by microorganisms during maturation. The chirospecific analysis of 3-hydroxybutan-2-one (8), 2-hydroxypentan-3-one (9), and 3-hydroxypentan-2-one (10) was achieved (Mosandl et al., 1990a) and their order of elution (8, 9) assigned with optically pure references (Fischer and Mosandl, 1990). Due to the vicinity of the asymmetric center and a carbonyl function, a racemization of 1,2-ketols seems to be possible. Nevertheless, the actual chirality evaluation of 1,2-ketols in food is achieved by enantioselective CGC. By use of MDGC and permethylated β-cyclodextrin as the chiral stationary phase the chiral 1,2-ketols 8–10 from the steam distillate of honey are stereoanalyzed: 8 (*R*, 45%; *S*, 55%); 9 (*R*, 60%; *S*, 40%); 10 (65 and 35%, chirality not identified as yet).

CONCLUSIONS

Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin is known to be an efficient chiral stationary phase that separates optical isomers of a wide variety by enantioselective inclusion gas chromatography. Using the column combination Carbowax 20M/permethylated β-cyclodextrin, multidimensional gas chromatography (MDGC) is proven to be a suitable method to stereoanalyze the mirror images

of 2-methylbutanoic acid, 2-alkan-2-yl acetates (1–3), (*Z*)-4-hepten-2-yl acetate (4), the chiral monoterpenes α-pinene (5), β-pinene (6), and limonene (7), and chiral 1,2-ketols (8–10) from food extracts and other complex matrices.

The described direct chirality evaluation is of considerable interest with respect to the validation of the natural origin of flavors.

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LITERATURE CITED

Chinghai, W.; Frank, H.; Guanghua, W.; Liangmo, Z.; Bayer, E.; Peichang, L. Determination of amino acid enantiomers by two-column gas chromatography with valveless column switching. *J. Chromatogr.* **1983**, *262*, 352.

Deger, W. Dissertation, University of Würzburg, 1988.

Fischer, K. Dissertation, University of Frankfurt, 1990.

Fischer, K.; Mosandl, A. *Z. Lebensm. Unters. Forsch.* **1990**, in press.

Fröhlich, O.; Huffer, M.; Schreier, P. Chirality Evaluation of 2-Heptanol and Z-4-Hepten-2-ol from Banana Fruit Using Multidimensional Gas Chromatography *Z. Naturforsch.* **1989**, *44C*, 555.

Gessner, M.; Deger, W.; Mosandl, A. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 417.

Guichard, E.; Souty, M. Comparison of the relative quantities of aroma compounds found in fresh apricot (Prunus armeniaca) from six different varieties. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 301.

Hener, U.; Kreis, P.; Mosandl, A. Chiral Compounds of Essential Oils IV. Enantiomer Distribution of α-Pinene, β-Pinene and Limonene in Essential Oils and Extracts; Part 2: Perfumes and Cosmetics. *Flavour Fragrance J.* **1991a**, *5*, 201.

Hener, U.; Kreis, P.; Mosandl, A. Chiral Compounds of Essential Oils VI. Enantiomer Distribution of α-Pinene, β-Pinene and Limonene in Essential Oils and Extracts; Part 3: Oils for Alcoholic Beverages and Seasonings. *Flavour Fragrance J.* **1991b**, in press.

Idstein, H.; Bauer, C.; Schreier, P. *Z. Lebensm. Unters. Forsch.* **1985**, *180*, 394.

König, W. A.; Lutz, S.; Colberg, C.; Schmidt, N.; Wenz, G.; von der Bey, E.; Mosandl, A.; Günther, C.; Kustermann, A. Cyclodextrins as Chiral Stationary Phases in Capillary Gas Chromatography, Part III: Hexakis(3-O-acetyl-2,6-di-O-pentyl)-α-cyclodextrins. *HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun.* **1988**, *11*, 621.

König, W. A.; Krebber, R.; Wenz, G. Enantioselective Capillary Gas Chromatography on the Basis of Host-Guest Interactions with Modified Cyclodextrins. *J. High Resolut. Chromatogr.* **1989a**, *12*, 641.

König, W. A.; Krebber, R.; Mischnick, P. Cyclodextrins as Chiral Stationary Phases in Capillary Gas Chromatography, Part V: Octakis(3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin. *J. High Resolut. Chromatogr.* **1989b**, *12*, 732.

König, W. A.; Krebber, R.; Evers, P.; Bruhn, G. Stereochemical Analysis of Constituents of Essential Oils and Flavor Compounds by Enantioselective Capillary Gas Chromatography. *J. High Resolut. Chromatogr.* **1990**, *13*, 328.

Kreis, P.; Hener, U.; Mosandl, A. *Dtsch. Apoth. Ztg.* **1990**, *130*, 985.

Kreis, P.; Hener, U.; Mosandl, A. *Dtsch. Lebensm. Rundsch.* **1991**, *87*, 8.

Mosandl, A.; Hener, U.; Hagenauer-Hener, U.; Kustermann, A. Stereoisomeric flavor compounds XXXII: Direct enantiomer separation of chiral γ-lactones from food and beverages by multidimensional gas chromatography. *J. High Resolut. Chromatogr.* **1989**, *12*, 532.

Mosandl, A.; Rettinger, K.; Fischer, K.; Schubert, V.; Schmarr, H.-G.; Maas, B. Stereoisomeric Flavor Compounds XLI: New Applications of Permethylated β-Cyclodextrin Phase in Chiral CGC-Analysis. *J. High Resolut. Chromatogr.* **1990a**, *13*, 382.

Mosandl, A.; Hener, U.; Kreis, P.; Schmarr, H.-G. Chiral Compounds of Essential Oils II. Enantiomer Distribution of α-Pinene, β-Pinene, and Limonene in Essential Oils and Extracts; Part 1: Rutaceae and Gramineae. *Flavour Fragrance J.* **1990b**, *5*, 193.

Nitz, S.; Kollmannsberger, H.; Drawert, F. Determination of Sensorial Active Trace Compounds by Multidimensional Gas Chromatography combined with Different Enrichment Techniques. *J. Chromatogr.* **1989**, *471*, 173.

Nowotny, H.-P.; Schmalzing, D.; Wistuba, D.; Schurig, V. Extending the Scope of Enantiomer Separation of Diluted Methylated β-Cyclodextrin Derivatives by High-Resolution Gas Chromatography. *J. High Resolut. Chromatogr.* **1989**, *12*, 383.

Rettinger, K. Dissertation, University of Frankfurt, 1991.

Rettinger, K.; Weber, B.; Mosandl, A. *Z. Lebensm. Unters. Forsch.* **1990**, *191*, 265.

Schomburg, G.; Husmann, H.; Hübinger, E.; König, W. A. Multidimensional Capillary Gas Chromatography-Enantiomeric Separations of Selected Cuts Using a Chiral Second Column. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1984**, *7*, 404.

Schubert, V. Dissertation, University of Frankfurt, 1991.

Schurig, V.; Nowotny, H.-P. Separation of Enantiomers on Diluted Permethylated β-Cyclodextrin by High-Resolution Gas Chromatography. *J. Chromatogr.* **1988**, *441*, 155.

Takeoka, G.; Flath, R. A.; Thomas, R. M.; Buttery, R. G.; Teranishi, R.; Güntert, M.; Lautamo, R.; Szejtli, J. Further Applications of Permethylated β-Cyclodextrin Capillary Gas Chromatographic Columns. *J. High Resolut. Chromatogr.* **1990**, *13*, 202.

Tressl, R.; Drawert, F.; Heimann, W.; Emberger, R. *Z. Naturforsch.* **1969**, *24B*, 781.

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Registry No. (*S*)-2-Methylbutanoic acid, 1730-91-2; (*R*)-2-methylbutanoic acid, 32231-50-8; (*S*)-2-pentanol, 26184-62-3; (*R*)-2-pentanol, 31087-44-2; (*S*)-2-hexanol, 52019-78-0; (*R*)-2-hexanol, 26549-24-6; (*S*)-2-heptanol, 6033-23-4; (*R*)-2-heptanol, 6033-24-5; (*S*)-(*Z*)-4-hepten-2-ol, 124753-75-9; (*R*)-(*Z*)-4-hepten-2-ol, 124753-74-8; (*S*)-α-pinene, 7785-26-4; (*R*)-α-pinene, 7785-70-8; (*R*)-β-pinene, 19902-08-0; (*S*)-β-pinene, 18172-67-3; (*S*)-limonene, 5989-54-8; (*R*)-limonene, 5989-27-5; (*R*)-3-hydroxy-2-butanone, 53584-56-8; (*S*)-3-hydroxy-2-butanone, 78183-56-9; (*R*)-2-hydroxy-3-pentanone, 113919-09-8; (*S*)-2-hydroxy-3-pentanone, 125948-63-2; (*S*)-3-hydroxy-2-pentanone, 132881-72-2; (*R*)-3-hydroxy-2-pentanone, 113919-08-7.