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Registry No. 2-Pentanone, 107-87-9; hexanal, 66-25-1; (*E*)-2-hexenal, 6728-26-3; 3-methylpentanol, 589-35-5; 2-methylbutanoic acid, 116-53-0; (*Z*)-3-hexenol, 928-96-1; ethylbenzene, 100-41-4; hexanol, 25917-35-5; benzaldehyde, 100-52-7; (*E*)-2-heptenal, 18829-55-5; oct-1-en-3-ol, 3391-86-4; 6-methylhept-5-en-2-one, 110-93-0; octanal, 124-13-0; myrcene, 123-35-3; phenylacetaldehyde, 122-78-1; limonene, 138-86-3; 2-phenylethanol, 60-12-8; nonanal, 124-19-6; (*E*)-2-nonenal, 18829-56-6; (*Z*)-3-hexenyl butanoate, 16491-36-4; decanal, 112-31-2; (*E,E*)-2,4-nonadienal, 5910-87-2; benzothiazole, 95-16-9; 1-tridecene, 2437-56-1; α-cubebene, 17699-14-8; β-damascenone, 23726-93-4; cyclosatirene, 22469-52-9; α-copaene, 3856-25-5; β-bourbonene, 5208-59-3; β-cubebene, 13744-15-5; 1-tetradecene, 1120-36-1; caryophyllene, 87-44-5; β-copaene, 18252-44-3; (*E*)-α-bergamotene, 13474-59-4; (*E*)-β-farnesene, 18794-84-8; humulene, 6753-98-6; β-santalene, 511-59-1; α-muurolene, 10208-80-7; germacrene D, 23986-74-5; β-selinene, 17066-67-0; 1-pentadecene, 13360-61-7; bicyclogermacrene, 24703-35-3; α-muurolene, 10208-80-7; γ-cadinene, 39029-41-9; calamenene, 483-77-2; δ-cadinene, 483-76-1; calacorene, 38599-17-6; (*E,Z,E*)-1,3,5,11-tridecatetraene-7,9-diyne, 63366-81-4; (*E,E,E*)-1,3,5,11-tridecatetraene-7,9-diyne, 17091-00-8; (*Z,E,E*)-1,3,5,11-tridecatetraene-7,9-diyne, 124604-43-9; (*Z,Z*)-1,8,11-heptadecatriene, 56134-03-3; (*Z,E*)-1,3,11-tridecatriene-5,7,9-triyne, 124604-44-0; (*Z,Z,Z*)-1,8,11,14-heptadecatetraene, 10482-53-8; (*Z*)-1,11-tridecadiene-3,5,7,9-tetrayne, 59950-58-2; (*E,E*)-1,3,11-tridecatriene-5,7,9-triyne, 50739-51-0; 1-heptadecene, 6765-39-5; (*E*)-1,11-tridecadiene-3,5,7,9-tetrayne, 26130-86-9; mint sulfide, 72445-42-2; (*Z*)-1,3-tridecadiene-5,7,9,11-tetrayne, 124604-45-1; (*E,E*)-1,3,5-tridecatriene-7,9,11-triyne, 6581-77-7; (*E*)-1,3-tridecadiene-5,7,9,11-tetrayne, 3760-28-9; 1-tridecene-3,5,7,9,11-pentayne, 2060-59-5.

Stereoisomeric Flavor Compounds. 33. Multidimensional Gas Chromatography Direct Enantiomer Separation of γ-Lactones from Fruits, Foods, and Beverages

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Chiral γ-lactones from the raw flavor extract of strawberries and of some commercially available fruit-containing foods and beverages were directly stereoanalyzed by multidimensional gas chromatography (MDGC), employing heart-cutting techniques from DB 1701 as the preseparation column onto heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin as the chiral stationary phase.

The influence of optical isomerism to odor quality is well appreciated (Russell and Hills, 1971; Friedman and Miller, 1971; Ohloff, 1986; Mosandl, 1982), and in our opinion, research on the structure–function relationships of flavor substances will become increasingly important.

Chirality evaluation is a convenient method to differentiate between flavor compounds of natural origin and synthetic racemates, if comprehensive data about optical purity and fruit-specific distribution are available (Mosandl et al., 1988; Gessner et al., 1988).

Recently we reported on the first direct chirospecific analysis of chiral γ-lactones from foods and other commercially available fruit-containing preparations by off-line coupling of HPLC with HRGC on modified chiral α-cyclodextrin as a suitable chiral stationary phase (Mosandl and Kustermann, 1989b). This paper reports on multidimensional gas chromatography (MDGC) (Schomburg et al., 1984) as a very sensitive method of high selectivity to differentiate γ-lactone mirror images from complex flavor extracts, using heart-cutting techniques from DB-1701 as the nonchiral preseparation col-

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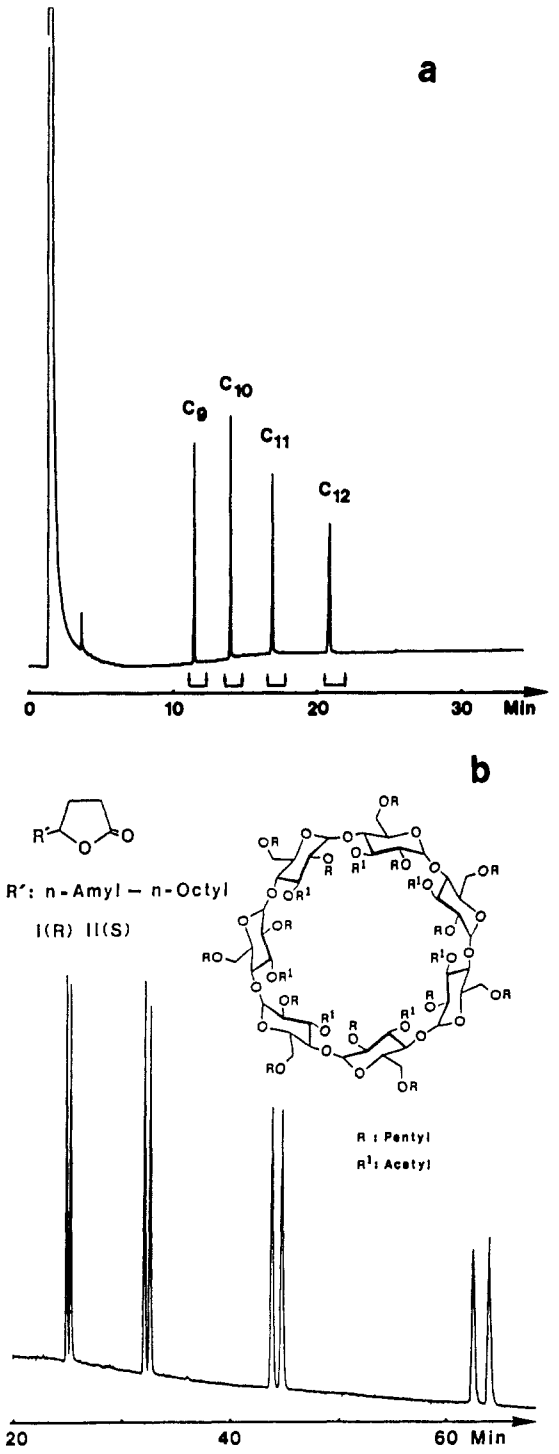

Figure 1. (a) Preseparation of racemic γ-nona- (C_9), γ-deca- (C_{10}), γ-undeca- (C_{11}), and γ-dodecalactone (C_{12}) as a standard mixture on DB-1701. Conditions: 0.9 bar of H_2; program, 140 °C, 2 min isothermal, 5 °C/min → 200 °C; back-flush after 33 min; elution intervals (↕), transferred onto the main column. (b) Transfer of racemic γ-nona- (C_9), γ-deca- (C_{10}), γ-undeca- (C_{11}), and γ-dodecalactone (C_{12}) from DB-1701 preseparation (a) onto the main column and base-line resolution with Lipodex D as the chiral stationary phase. Conditions: 0.75 bar of H_2; program, 140 °C, 10 min isothermal, 5 °C/min → 170 °C; order of elution assigned with optically pure references (Günther, 1988; Mosandl and Günther, 1989).

umn and transfer onto heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin as the appropriate chiral stationary phase.

EXPERIMENTAL SECTION

Optically Pure References. The structure and properties of γ-lactone enantiomers are reported in previous papers

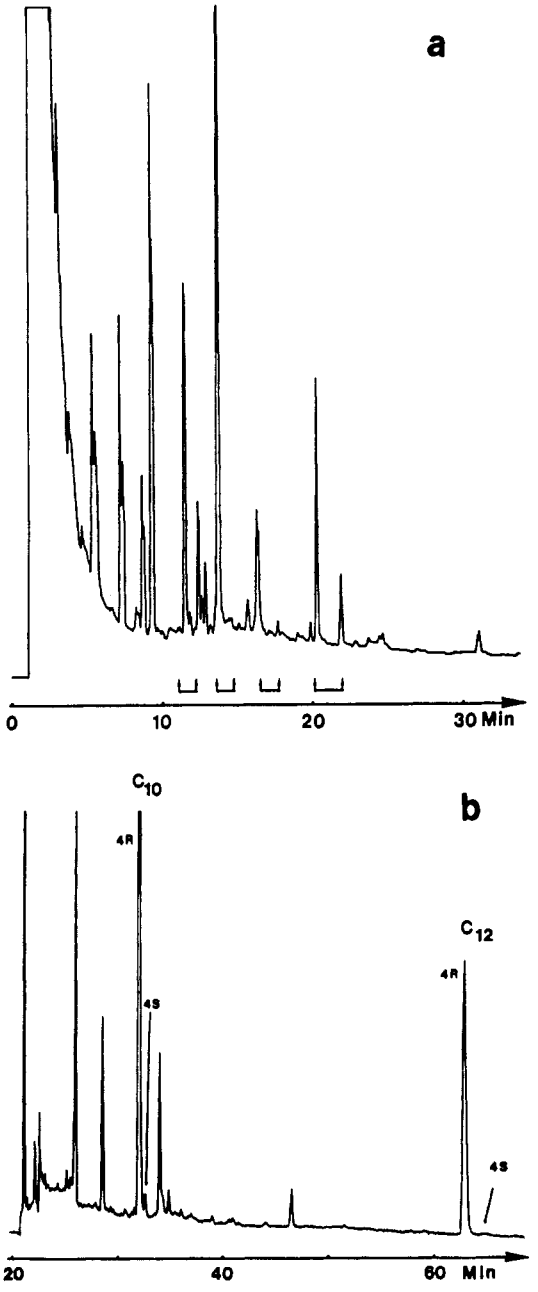

Figure 2. (a) Raw strawberry extract, preseparated with DB-1701. Conditions: see Figure 1a. (b) Chirality evaluation of γ-deca- (C_{10}) and γ-dodecalactone (C_{12}) from strawberries transferred from DB-1701 preseparation (a) onto the main column. Conditions: see Figure 1b.

(Günther, 1988; Mosandl and Günther, 1989a). The order of elution from the modified chiral α-cyclodextrin and β-cyclodextrin phase is assigned by optically pure references: I (*R*), II (*S*) (Günther, 1988; König et al., 1988; Mosandl et al., 1989a).

Instrumentation. 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, splitless, 0.6 min; injection temperature, 200 °C, detection temperature, 220 °C.

Separation conditions: carrier gas, H_2; preseparation pressure, P_A = 0.9 bar; main separation pressure, P_M = 0.75 bar.

Preseparation. A fused silica retention gap, 10 m × 0.25 mm (i.d.), deactivated according to Grob (1987), coupled with a nonchiral DB-1701 (86% dimethyl–7% cyanopropyl–7% phenylpolysiloxane) chemically bonded fused silica column, 15 m × 0.25 mm (i.d.) with 1-μm film thickness (J&W Scientific),

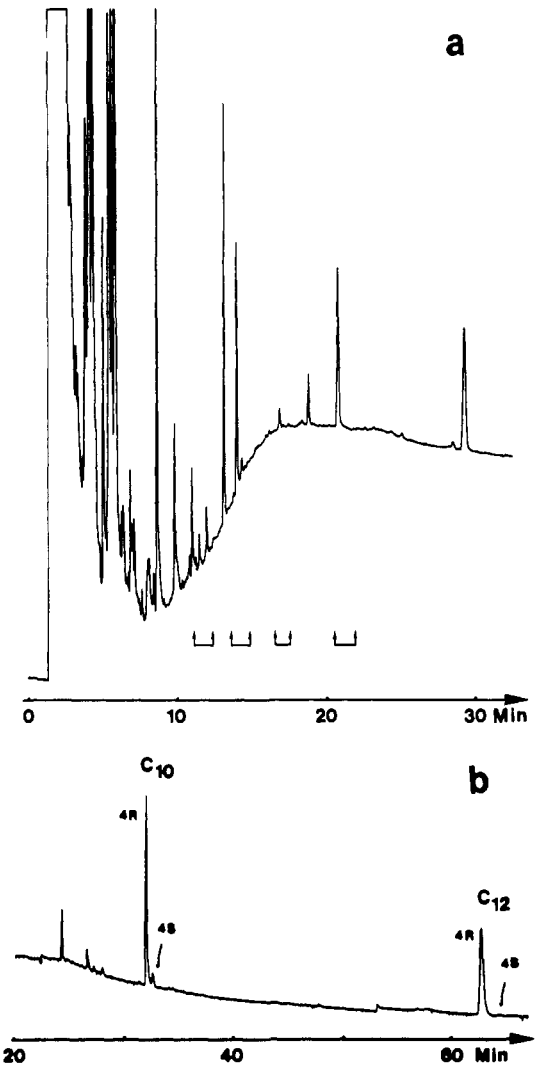

Figure 3. (a) Dichloromethane extract from apricot brandy, purchased from the market, analyzed on a DB-1701 precolumn. Conditions: see Figure 1a. (b) Chirospecific analysis of γ-deca- (C_{10}) and γ-dodecalactone (C_{12}) from the distillate of apricot brandy (a), transferred to stereoanalysis with Lipodex D. Conditions: see Figure 1b.

was used. Program: 140 °C, 2 min isothermal, 5 °C/min → 200 °C, back-flush after 33 min.

Main Separation. A pyrex glass capillary column, 38 m × 0.2 mm (i.d.), coated with heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin (Lipodex D) as the chiral stationary phase (König et al., 1988) for the direct resolution of optically active γ-lactones was used (Mosandl and Kustermann, 1989b). Program: 140 °C, 10 min isothermal, 5 °C/min → 170 °C.

Sample Preparation. *General Procedure.* The quantity of foods to be analyzed depends on their genuine concentration of γ-lactones. Solid samples, e.g., 1–2 kg of freshly harvested strawberries, are homogenized, diluted with water, and centrifuged at 5000*g* for 30 min. The supernatant is exhaustively extracted with dichloromethane or pentane–dichloromethane on the apparatus of Likens and Nickerson or modified techniques. For standard controlled extraction, a suitable, noninterferring γ-lactone (1 ppm) as an internal standard is recommended.

The organic layer is dried over Na_2SO_4 (anhydrous) and concentrated to about 25 mL, on a Vigreux column. If preservatives (sorbic acid or benzoic acid) are detectable, these acids are removed by Na_2CO_3 (5%). After being dried and concentrated to 0.5 mL, the solution is ready to use for MDGC.

Nonalcoholic Liquid Samples. Fruit juices and other fruit-containing beverages (1–2 L) are diluted with water and worked up in a similar manner.

Alcoholic Beverages. They are diluted with water to an alcohol content of about 20% before extraction with dichloromethane or pentane–dichloromethane. Liquors are distilled first; subsequently the diluted distillate is worked up.

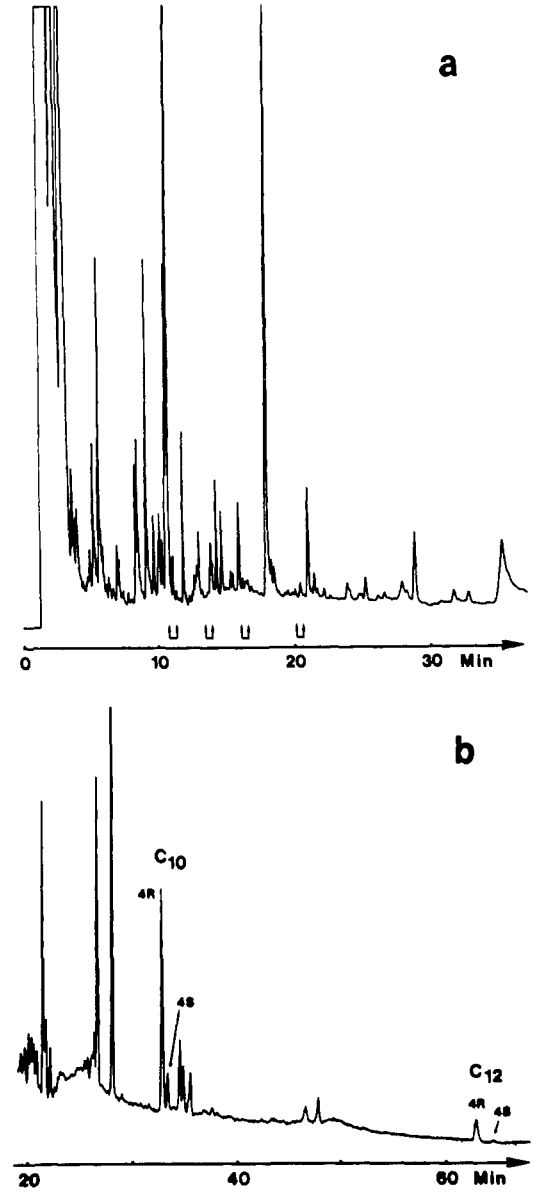

Figure 4. (a) Dichloromethane extract from multivitamin fruit juice, purchased from the market and analyzed on a DB-1701 precolumn. Conditions: see Figure 1a. (b) Stereodifferentiation of γ-deca- (C_{10}) and γ-dodecalactone (C_{12}) from multivitamin fruit juice, transferred from a DB-1701 precolumn (a) onto Lipodex D as the chiral stationary phase. Conditions: see Figure 1b.

RESULTS AND DISCUSSION

Despite the importance of γ-lactones in flavors of natural origin, their stereochemical structure–function relationships have remained unknown until now. Recently, the chiroptical and sensory properties of their mirror images were described (Günther, 1988; Mosandl and Günther, 1989a) and the first chirospecific methods (via the diastereomeric esters) evaluated (Mosandl et al., 1988).

Meanwhile, the analytical γ-lactone enantiomer separation has been achieved and we have reported on the first direct chirospecific analysis of γ-lactones from foods and some other commercially available fruit-containing preparations by off-line coupling of HPLC with HRGC on modified chiral α(β)-cyclodextrins as suitable chiral stationary phases (Mosandl and Kustermann, 1989b; Mosandl et al., 1989b).

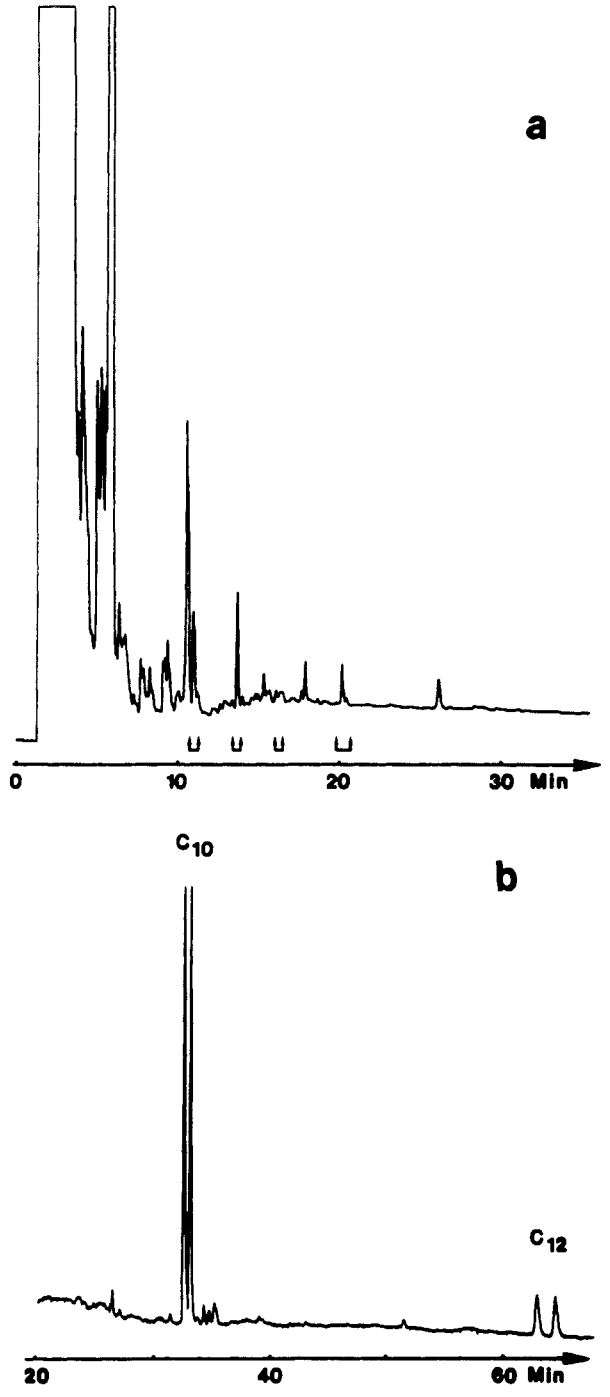

Figure 5. (a) Dichloromethane extract from an orange/maracuja fruit nectar, purchased from the market and analyzed with a DB-1701 precolumn. Conditions: see Figure 1a. (b) Enantiodifferentiation of γ-deca- (C_{10}) and γ-dodecalactone (C_{12}) from an orange/maracuja fruit nectar (a), transferred onto the chiral main column Lipodex D. Conditions: see Figure 1b.

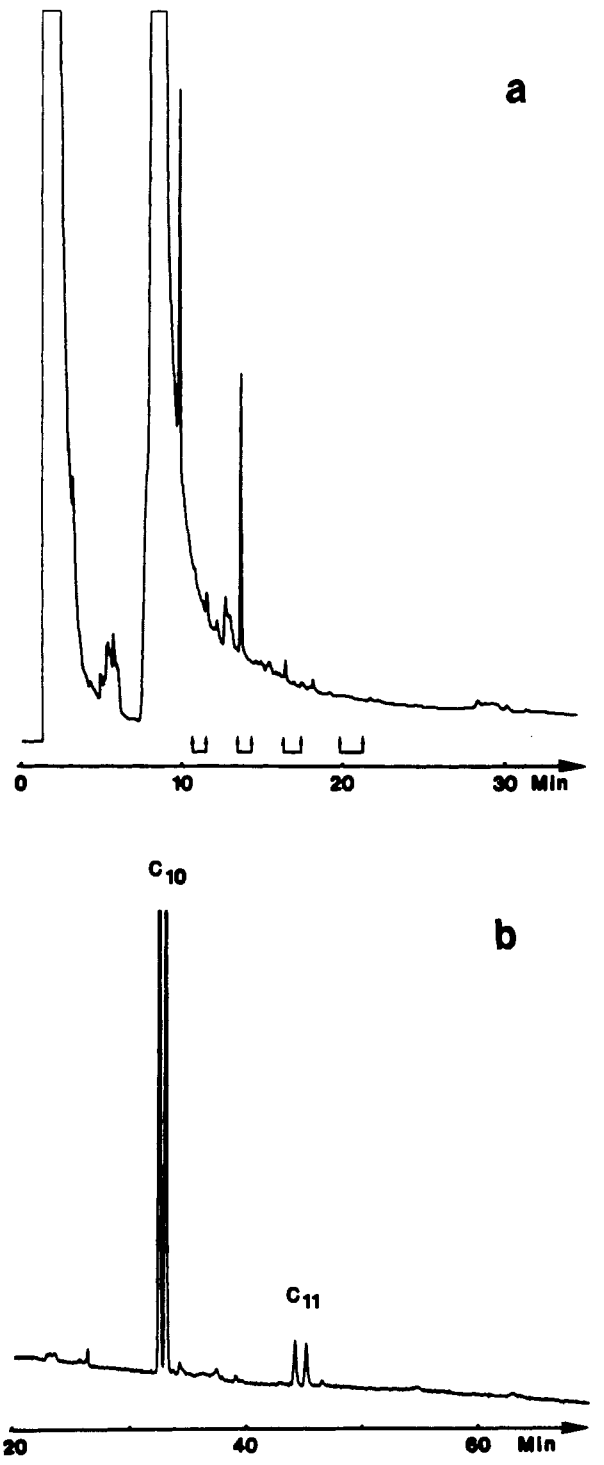

Figure 6. (a) Dichloromethane extract from a fruit preparation with passion fruits, analyzed with nonchiral precolumn DB-1701. Conditions: see Figure 1a. (b) Racemic γ-deca- (C_{10}) and racemic γ-undecalactone (C_{11}), stereoanalyzed after transfer from DB-1701 (a) onto Lipodex D. Conditions: see Figure 1b.

However, due to the complexity of natural flavor extracts, an effective preseparation has to be assumed as an indispensable prerequisite for the direct enantiodifferentiation of γ-lactones from flavor matrices. MDGC is demonstrated with racemic mixtures of γ-nona- (C_9), γ-deca- (C_{10}), γ-undeca- (C_{11}), and γ-dodecalactone (C_{12}) by DB-1701 preseparation (Figure 1a) and transfer onto the main column, coated with heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin as the chiral stationary phase. Base-line resolution is achieved in all four cases (Figure 1b).

Due to their enzymatic pathways, chiral aroma compounds from fruits and other natural sources are characterized by definite and fruit-specific distribution of their enantiomers. From freshly harvested strawberries, γ-decalactone and γ-dodecalactone are detected in a 70:30 ratio and with rather high optical purity in favor of the 4*R*-configurated γ-lactones: γ-C_{10}, 4*R*, >98% ee; γ-C_{12}, 4*R*, >99% ee (cf. Figure 2a,b).

The chirospecific analyses of γ-decalactone and γ-dodecalactone from the distillate of an apricot brandy indicate optical purities similar to those of strawberries (Figure 3a,b).

The distribution of γ-deca- and γ-dodecalactone enan-

tiomers, isolated from a commercially available multivitamin fruit juice, is documented in Figure 4a,b.

On the other hand, the natural occurrence of racemic γ-lactones has not yet been observed. Therefore, the detection of a racemic γ-lactone from fruit-containing foods indicates the addition of synthetic flavorings, while chiral aroma compounds from natural sources reflect the fruit-specific distribution of their enantiomers (Mosandl et al., 1988, 1989b). By MDGC analysis of γ-lactones from an orange/maracuja nectar (Figure 5a,b) and from a fruit preparation with passion fruits (Figure 6a,b), the addition of racemic γ-lactones is identified, in contrast to legal regulations of German food law.

CONCLUSION

Multidimensional gas chromatography employing heart-cutting techniques from DB-1701 as the preseparation column onto heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)-β-cyclodextrin is proven to be a powerful method for the direct enantiomer separation of chiral γ-lactones from complex multicomponent mixtures without any further cleanup or derivatization procedures. The described MDGC technique is of considerable interest with regard to the importance of chiral γ-lactones as key aroma compounds and the legal interpretation of their addition to foods and beverages.

ACKNOWLEDGMENT

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Registry No. (4*R*)-γ-Decalactone, 107797-26-2; (4*R*)-γ-dodecalactone, 69830-91-7; (4*S*)-γ-dodecalactone, 69830-92-8; (4*S*)-γ-decalactone, 107797-27-3; γ-nonalactone, 104-61-0; γ-decalactone, 706-14-9; γ-undecalactone, 104-67-6; γ-dodecalactone, 2305-05-7.