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Note

Determination of the enantiomeric composition of γ-lactones in complex natural matrices using multidimensional capillary gas chromatography

ALEXANDER BERNREUTHER
Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, D-8700 Würzburg (F.R.G.)
NORBERT CHRISTOPH
Landesuntersuchungsamt für das Gesundheitswesen Nordbayern, Theaterstr. 23, D-8700 Würzburg (F.R.G.)
and
PETER SCHREIER*
Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, D-8700 Würzburg (F.R.G.)
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It is generally recognized that chiral discrimination is an essential principle of odour perception[1], and the determination of enantiomeric composition of aroma compounds is therefore very important in flavour analysis[2].

γ-Lactones are widely used as intermediates in the synthesis of natural products and are important, widespread aroma compounds[3,4]. Recently, sensory characteristics of several optically pure γ-lactones have been described[5]. Owing to the importance of this class of compounds in flavour chemistry, there have been a number of publications dealing with their chiral analysis. Recently, direct enantiomer separation of γ-lactones on chiral stationary phases has been described, *e.g.*, using both capillary (high-resolution) gas chromatography (HRGC)[6] and high-performance liquid chromatography (HPLC)[7,8]. However, results for the chiral evaluation of naturally occurring γ-lactones in plant tissues, *e.g.*, fruits, are scarce[9–12] owing to the difficulties in determining their enantiomeric composition in a complex natural matrix. In order to avoid laborious sample preparation and preseparation, multidimensional capillary GC (MDGC) has been recommended[13,14]. Extending our recent work on the chirality evaluation of secondary alcohols from banana fruit using MDGC with achiral and chiral columns[15], this paper reports the use of MDGC for the determination of the enantiomeric composition of γ-lactones in various fruits.

EXPERIMENTAL

Fruits

Fresh mangoes (from Venezuela) and yellow passion fruits (from Columbia) and deep-frozen apricots, peaches, raspberries and strawberries (all purchased in the local market) were used.

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Sample preparation

Deep-frozen fruits were thawed, stone fruits were stoned and passion fruits were peeled. After addition of 1 l of distilled water to 2.5 kg of fruit pulp and homogenization in a Braun blender (3 min), centrifugation (40 min, 3000 *g*) was carried out. The supernatants were continuously extracted over 36 h using pentane–dichloromethane (2:1) (40°C). The extracts were dried over anhydrous sodium sulphate and carefully concentrated to approximately 0.2 ml using a Vigreux column (45°C).

Optically pure reference substances

(*R*)- and (*S*)-4-alkylated γ-lactones were obtained from racemic references (γ-pentalactone to γ-dodecalactone; Roth, Karlsruhe, F.R.G.) by semi-preparative HPLC separation on a Hibar Chiraspher column (RT 250-4; 250 × 4 mm I.D.; particle diameter 5 μm; Merck, Darmstadt, F.R.G.) as recently described[8].

Capillary gas chromatography–mass spectrometry (HRGC–MS)

A Hewlett-Packard HP 5890A gas chromatograph coupled by an open-split interface to an HP 5970B mass-selective detector with an HP 300 data system was used. The apparatus was equipped with a J & W DB-Wax capillary column (30 m × 0.32 mm I.D.; film thickness 0.25 μm). Splitless injection [200°C; split opened (1:13) after 0.3 min] was employed. The column temperature was programmed from 40 to 250°C at 3.5°C/min. The carrier gas was helium at 1.4 ml/min, the temperature of the ion source and all connecting parts was 200°C and the electron energy was 70 eV. The selected ion monitoring (SIM) mode using *m/z* 85, 56 and 100 for γ-lactone detection was used.

Multidimensional gas chromatography (MDGC)

A Siemens Sichromat 2 double oven gas chromatograph with split injection (250°C; 1:55) and flame ionization detectors on ovens 1 and 2 (both at 250°C) was used. Preseparation was achieved in oven 1 on a BC SE-54 fused-silica capillary column (25 m × 0.25 mm I.D.; film thickness 0.2 μm). The temperature was programmed from 80 to 280°C at 5°C/min and held isothermally at 280°C for 20 min. A "live" switching device[16] in oven 1 was used to perform effluent cuts on column 2 in oven 2 [Pyrex glass capillary, Lipodex B (Macherey, Nagel & Co.), 25 m × 0.25 mm I.D.). The temperature was isothermal at 100°C for 10 min, then programmed from 100 to 180°C at 2°C/min and held isothermally at 180°C for 10 min. Cuts of 30 s were selected for each γ-lactone separately. Nitrogen was used as the carrier gas at 0.42 ml/min in oven 1 and 0.67 ml/min in oven 2. The flow-rates for the detector gases were 27 and 31 ml/min of hydrogen and 268 and 280 ml/min of air for ovens 1 and 2, respectively. Injection volumes of 0.5–2 μl were used.

Results of qualitative analyses were verified by comparison of MDGC retention data for authentic racemic and optically pure γ-lactones.

RESULTS AND DISCUSSION

First, the high capacity of Lipodex B [hexakis(3-O-acetyl-2,6-di-O-pentyl)-α-cyclodextrin] for the enantioseparation of γ-lactones[17] was confirmed under MDGC conditions. The α values obtained are given in Table I. In accordance with recent

TABLE I

SEPARATION FACTORS OBTAINED FOR HOMOLOGOUS CHIRAL 4-ALKYLATED γ-LACTONES (ALKYL CHAIN LENGTHS FROM C_1 TO C_8) UNDER MDGC CONDITIONS (SE-54/LIPODEX B)

γ-Lactone	*α-Value*	*γ-Lactone*	*α-Value*
γ-Pentalactone	1.154	γ-Nonalactone	1.028
γ-Hexalactone	1.126	γ-Decalactone	1.023
γ-Heptalactone	1.046	γ-Undecalactone	1.020
γ-Octalactone	1.030	γ-Dodecalactone	1.022

information[6], the order of elution, established with optically pure reference compounds, was (*R*) before (*S*). A detection limit of approximately 1 μg/ml was calculated.

In the aroma extracts obtained from the fruits studied (apricot, mango, passion fruit, peach, raspberry and strawberry), the occurrence of γ-lactones was checked by HRGC–MS using SIM. In MDGC analyses, their identities were verified by comparison of their HRGC retention data on two columns using authentic racemic and optically pure reference compounds. For the enantiomeric differentiation of natural γ-lactones on the chiral column, for each γ-lactone detected in the fruits a 30-s cut was performed according to the retention time. A representative example is shown in Fig. 1, in which the preseparation of peach volatiles on SE-54 without cutting and the enantiomeric separation of γ-decalactone on Lipodex B with cutting are outlined. All γ-lactones present in the above-mentioned fruits were investigated in a similar manner. The results are summarized in Table II.

For the composition of γ-lactones in the fruits studied, earlier elaborated data from the TNO list[18] were confirmed. However, in apricot γ-penta- and γ-heptalactone and in strawberry γ-penta-, γ-nona- and γ-undecalactone were additionally detected in trace amounts for the first time.

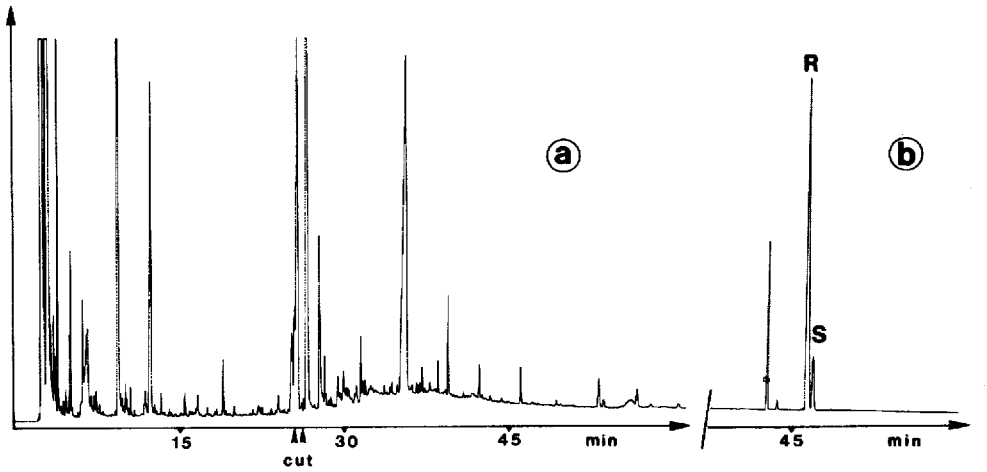

Fig. 1. MDGC separation of peach volatiles. (a) Preseparation on SE-54. (b) Enantiomeric separation of γ-decalactone on Lipodex B with cutting (25.5–26.0 min). See Experimental for details.

TABLE II

OCCURRENCE AND ENANTIOMERIC COMPOSITION OF CHIRAL 4-ALKYLATED γ-LACTONES IN DIFFERENT FRUITS

γ-Lactone	*Fruit*[a]	*(R)- (%)*	*(S)- (%)*	*Enantiomeric excess (%)*
γ-Pentalactone	AP	78.6	21.4	(*R*)-57.2
	MA	tr[b]	tr	–
	PE	73.4	26.6	(*R*)-46.8
	ST	40.1	59.9	(*S*)-19.8
γ-Hexalactone	AP	90.2	9.8	(*R*)-80.4
	MA	26.4	73.6	(*S*)-47.2
	PA	65.5	34.5	(*R*)-31.0
	PE	90.2	9.8	(*R*)-80.4
	RA	31.9	68.1	(*S*)-36.2
	ST	41.7	58.3	(*S*)-16.6
γ-Heptalactone	AP	81.8	18.2	(*R*)-63.6
	MA	tr	tr	–
	PA	32.4	67.6	(*S*)-35.2
	PE	33.5	66.5	(*S*)-33.0
	ST	34.2	65.8	(*S*)-31.6
γ-Octalactone	AP	89.1	10.9	(*R*)-78.2
	MA	53.4	46.6	(*R*)-6.8
	PA	59.1	40.9	(*R*)-18.2
	PE	86.8	13.2	(*R*)-73.5
	RA	39.9	60.1	(*S*)-20.2
	ST	66.2	33.8	(*R*)-32.4
γ-Nonalactone	AP	83.7	16.3	(*R*)-67.4
	MA	73.3	26.7	(*R*)-46.6
	PA	92.6	7.4	(*R*)-85.2
	PE	84.6	15.4	(*R*)-69.2
	ST	63.6	36.4	(*R*)-27.2
γ-Decalactone	AP	94.3	5.7	(*R*)-88.6
	MA	65.8	34.2	(*R*)-31.6
	PA	91.3	8.7	(*R*)-82.6
	PE	87.4	12.6	(*R*)-74.8
	ST	97.9	2.1	(*R*)-95.9
γ-Undecalactone	PE	81.2	18.8	(*R*)-62.4
	ST	tr	tr	–
γ-Dodecalactone	AP	100.0	0.0	(*R*)-100.0
	MA	100.0	0.0	(*R*)-100.0
	PA	97.6	2.4	(*R*)-95.2
	PE	96.3	3.7	(*R*)-92.6
	ST	98.3	1.7	(*R*)-96.6

[a] AP = apricot; MA = mango; PA = passion fruit; PE = peach; RA = raspberry; ST = strawberry.

[b] tr = Traces (enantiomeric discrimination not possible).

Concerning the enantiomeric distribution of γ-lactones in fruits, only a few studies have been carried out, *e.g.*, on nectarine[10], strawberry[11], peach[12] and mango[19]. Except for the enantiomeric composition of γ-octalactone in mango, the

previous findings were confirmed, *i.e.*, the occurrence of optically pure γ-deca- and γ-dodecalactone in strawberry, with an (*R*)- to (*S*)- ratio of approximately 88:12, and prevalence of the (*R*)-enantiomer of γ-decalactone in peach and mango. The discrepancy found for γ-octalactone in mango may be caused by varietal differences.

In general, in all fruits, the enantioselectivity increased with increasing chain length of γ-lactones (Table II). Whereas γ-dodecalactone was found to be present in approximately pure optical (*R*)-form in the fruits under study, shorter chained γ-lactones such as γ-pentalactone to γ-octalactone mainly showed large variations in the enantiomeric compositions. The biogenetic reason for this fact is not clear. At least two different enzyme systems or pathways in fruits, which potentially compete and lead to different enantiomeric ratios of γ-lactones, can be assumed[10].

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