Enantiodifferentiation of γ- and δ-Lactones by Gas Chromatographic Separation of Diastereomeric Carbamoyloxy Carboxamide Derivatives

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Enantiodifferentiation of chiral γ- and δ-lactones has been achieved by capillary gas chromatographic separation of diastereomeric 4- and 5-[(*R*)-[(1-phenylethyl)carbamoyl]oxy] *N*-butylcarboxamides. The derivatization procedure involves (1) ring-opening of lactones to hydroxycarboxamides by heating with butylamine and (2) subsequent conversion to diastereomeric carbamates by reaction with (*R*)-(+)-1-phenylethyl isocyanate. High separation factors were determined for the complete series of C_5–C_{12} γ-lactones and C_6–C_{12} δ-lactones, naturally occurring (trace) constituents of many fruits and vegetables.

Due to their pronounced sensory properties, 4- and 5-alkanolides, also termed γ- and δ-lactones, play important roles as flavor constituents of various foods (Maga, 1976). Increasing interest in the chirality evaluation of these compounds has been stimulated by the application of sensitive capillary gas chromatographic techniques for the determination of enantiomeric compositions of chiral aroma components occurring in natural systems at trace levels (Tressl and Engel, 1984, 1985; Tressl et al., 1985).

Direct separation of lactone enantiomers on optically active stationary phases is the most elegant approach. However, the practical use of Chira-Metal stationary phases is limited by long retention times for the higher lactone homologues (Schurig, 1988). Excellent results have been obtained by using modified cyclodextrin as stationary phase (König et al., 1988), but resolution of enantiomers has only been reported for γ-lactones.

The separation of diastereomeric derivatives on commonly available, achiral capillary columns is a useful alternative. Different procedures to convert lactones into intermediates with reactive functional groups and various derivatization reagents have been explored. Methyl and ethyl 4- and 5-hydroxy carboxylic acid esters, derived from γ- and δ-lactones, have been derivatized with (*R*)-(+)-1-phenylethyl isocyanate (Tressl et al., 1985). Ring-opening of lactones to hydroxy carboxylic acid isopropyl esters has been combined with conversion to diastereomeric esters of (*S*)-*O*-acetyllactic acid (Mosandl et al., 1987; Krammer et al., 1988; Feuerbach et al., 1988) and (*S*)-tetrahydro-5-oxo-2-furancarboxylic acid (Gessner et al., 1988). Reduction of γ- and δ-lactones with lithium aluminum hydride leads to 1,4- and 1,5-diols, respectively; their diastereomeric diesters of (*S*)-*O*-acyllactic acids (Deger et al., 1988) and their bis[(*R*)-1-phenylethyl] carbamates (Engel et al., 1989) could be separated by capillary GC.

A general drawback of all these procedures is the strong effect of lactone structure and chain length on the separation factors of diastereomeric derivatives. As a consequence, none of the methods alone is applicable to a comprehensive investigation of the broad spectrum of lactones of different chain lengths occurring in foods.

This paper presents an alternative procedure involving the conversion of lactones to hydroxy carboxamides and subsequent derivatization with (*R*)-(+)-1-phenylethyl isocyanate. Gas chromatographic separation factors obtained for the diastereoisomers are high enough to investigate the chirality of the complete series of C_5–C_{12} γ-lactones and C_6–C_{12} δ-lactones, those members of the class of lactones that are of special interest as constituents of fruits and vegetables.

EXPERIMENTAL SECTION

Derivatization. (*a*) *Formation of 4- and 5-Hydroxy Carboxamides.* In a screw-capped reaction vial, 0.5 μL of equimolar mixtures of either C_5–C_{12} γ-lactones or C_6–C_{12} δ-lactones and 10 μL of butylamine were heated at 80 °C for 5 h. The reaction mixture was dissolved in chloroform (40 mL) and transferred to a separatory funnel. After the solution was washed with 1 N HCl (1 mL), 5% $NaHCO_3$ solution (1 mL), and H_2O (1 mL) and dried with sodium sulfate, the solvent was evaporated. The residue was dissolved in 200 μL of diethyl ether and retransferred to a reaction vial.

(*b*) *Conversion to Diastereomeric Carbamates.* After removal of the ether by using a stream of nitrogen, 25 μL of toluene containing 1% (dimethylamino)pyridine (DMAP) and 2 μL of (*R*)-(+)-1-phenylethyl isocyanate (PEIC) were added. After 24 h at 60 °C the mixture was diluted with 250 μL of methylene chloride and subjected to GC analysis.

Capillary Gas Chromatography. A DB 210 column (J&W Scientific, Inc.; 30 m × 0.32 mm (i.d.), film thickness 0.25 μm) was installed in a Carlo Erba Fractovap Series 2150 gas chromatograph, equipped with split injection (1:25) and FID. Conditions: injector temperature, 230 °C; detector temperature, 275 °C; carrier gas, hydrogen; 0.85 bar; *u*(225 °C), 55 cm/s. The column was held at 220 °C for 5 min and then programmed to 240 °C with a rate of 1 °C/min.

Capillary Gas Chromatography–Mass Spectrometry. The DB 210 fused silica column was inserted directly into the ion source of a Finnigan MAT 4500 series quadrupole gas chromatograph–mass spectrometer equipped with an Incos data system. Conditions: ionization voltage, 70 eV; ion source temperature, 180 °C; speed, 1 scan/s; mass range (*m*/*e*), 33–500.

Chemicals. (*R*)-(+)-1-Phenylethyl isocyanate and 4-(dimethylamino)pyridine were purchased from Fluka, Neu-Ulm, West Germany. Racemic γ- and δ-lactones were gifts from Haarmann & Reimer, Holzminden, West Germany, and Firmenich, Geneva, Switzerland. Optically pure and enriched γ- and δ-lactones were obtained by baker's yeast catalyzed reductions of 4- and 5-oxo acids (Utaka et al., 1987; Gessner et al., 1987) and by liquid chromatographic resolution of lactone enantiomers on cellulose triacetate (Francotte and Lohmann, 1987). All solvents were redistilled before use.

RESULTS AND DISCUSSION

Diastereomeric derivatives of γ- and δ-lactones were obtained after (1) ring-opening by heating with butylamine and (2) conversion of the formed 4- and 5-

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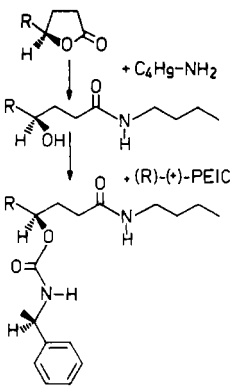

Figure 1. Conversion of γ-lactones to 4-[(*R*)-[(1-phenylethyl)carbamoyl]oxy] *N*-butylcarboxamides.

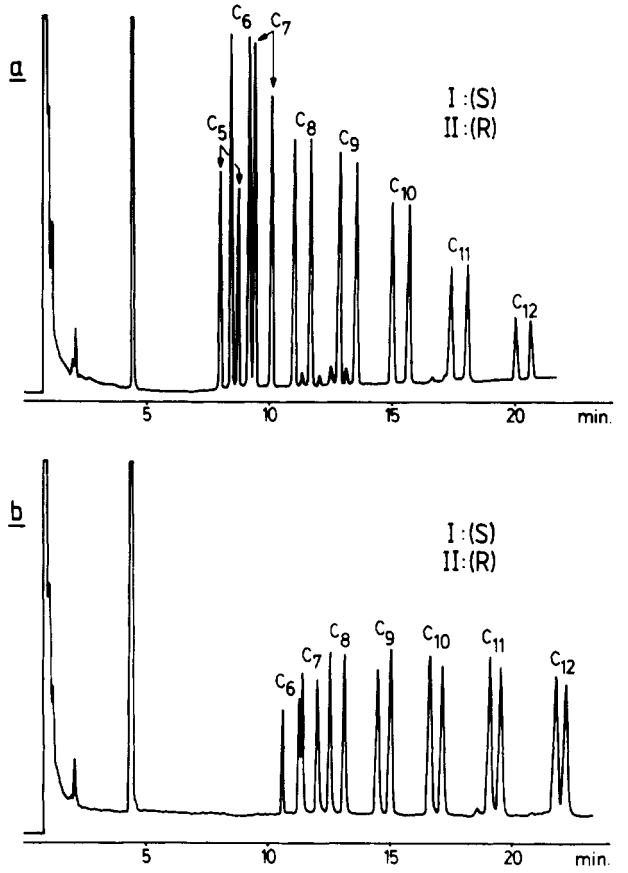

Figure 2. Capillary gas chromatographic separation of 4- and 5-[(*R*)-[(1-phenylethyl)carbamoyl]oxy] *N*-butylcarboxamides derived from γ-lactones (a) and δ-lactones (b) (for conditions see the Experimental Section).

hydroxy *N*-butyl carboxamides to carbamates by reaction with (*R*)-(+)-1-phenylethyl isocyanate (Figure 1). Capillary gas chromatographic separation of diastereomeric 4- and 5-[(*R*)-[(1-phenylethyl)carbamoyl]oxy] *N*-butylcarboxamides obtained from a homologous series of racemic C_5–C_{12} γ-lactones and C_6–C_{12} δ-lactones is shown in Figure 2.

Enantiomers of 2-substituted butyrolactones could be resolved by liquid chromatography after conversion to diastereomeric 4-hydroxybutyramides (Helmchen et al., 1979). Separation factors, however, decreased drastically when the asymmetric center of the lactones was shifted from α- to γ-position related to the carbonyl group (Helmchen and Nill, 1979). Ring-opening of homomevalono- and mevalonolactone by treatment with 1-(+)-α-(1′-naphthyl)ethylamine and subsequent esterification with (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid also led to diastereoisomers that could be separated by liquid chromatography (Bergot et al., 1979). To obtain derivatives more suitable for capillary GC analysis in the present study, butylamine was chosen to form hydroxy carboxamides; (*R*)-(+)-1-phenylethyl isocyanate was used to introduce the second asymmetric center. Similar derivatives—(isopropylcarbamoyl)oxy *N*-isopropylcarboxamides (Benecke and König, 1982; Frank et al., 1983) and (*tert*-butylcarbamoyl)oxy *N*-*tert*-butylcarboxamides (König et al., 1983)—have been described as useful for separation of enantiomers of 2- and 3-hydroxy acid esters on chiral stationary phases.

The conversion of lactones to *N*-butylamides proceeded quantitatively without need of a catalyst. For the second derivatization step, catalytic amounts of 4-(dimethylamino)pyridine were added (Höfle et al., 1978; Rudmann and Aldrich, 1987). DMAP not only led to quantitative formation of carbamates at moderate temperature, but also prevented partial recyclizations of the hydroxy carboxamides to lactones observed in experiments without this catalyst.

The identities of derivatives were confirmed by gas chromatography–mass spectrometry. Table I presents mass spectral data of (*R*)-[(1-phenylethyl)carbamoyl]oxy *N*-butylcarboxamides derived from *R*-configurated γ- and δ-lactones. The spectra of derivatives obtained from the corresponding *S* enantiomers were very similar and showed only slight differences in intensities.

The first major peak (t_r = 4.4 min; Kovats index 3070) in the chromatograms shown in Figure 2a,b was identified as *N*,*N*-bis[(*R*)-1-phenylethyl]urea, a byproduct formed from (*R*)-1-phenylethyl isocyanate (Koppenhoefer and Allmendinger, 1987).

The gas chromatographic order of elution of derivatives was determined by means of optically enriched reference compounds. Within the homologous series of C_5–C_{12} γ-lactones and C_6–C_{12} δ-lactones, the order of elution of diastereoisomers remained constant; carbamoyloxy carboxamides obtained from (*S*)-lactones are eluted before those derived from the corresponding *R* enantiomers.

Optically pure reference compounds were used to demonstrate that the derivatization proceeds without racemization. Figure 3 presents the gas chromatographic separations of carbamoyl carboxamides derived from (*R*,*S*)- and (*S*)-4-heptanolide, respectively. The optical purity (>99.9% ee) of the employed reference lactone, obtained by liquid chromatography on cellulose triacetate (Francotte and Lohmann, 1987), had been determined by conversion to the corresponding 1,4-diol and subsequent derivatization with (*S*)-*O*-acetyllactyl chloride (Mosandl et al., 1987). The minor peak detectable for the second diastereoisomer in Figure 3 is not due to a partial racemization but reflects the content (1.6%) of *S* enantiomer in the batch of (*R*)-(+)-1-phenylethyl isocyanate used for this experiment. This optical impurity had been determined before by comparison of results obtained by derivatization of optically pure secondary alcohols with (*R*)-(+)-PEIC and (*S*)-*O*-acetyllactyl chloride.

This example demonstrates one of the general disadvantages of gas chromatographic chirality determinations via diastereomeric derivatives: The optical purities of commercially available derivatization reagents may vary, and they have to be checked before accurate determinations of high enantiomeric excesses can be carried out.

Separation factors of the diastereomeric carbamoyl carboxamide pairs are listed in Table II. A comparison with α-values obtained for PEIC derivatives of 4- and 5-hydroxy carboxylic acid esters (Tressl et al., 1985) demon-

Table I. Mass Spectral Data of 4- and 5-[(*R*)-[(1-Phenylethyl)carbamoyl]oxy] *N*-Butylcarboxamides Derived from the *R* Enantiomers of γ- and δ-Lactones

lactone	MW	*m/e* (relative intensity, %)
		γ-Lactones
4-pentanolide	320	156 (100), 57 (76), 105 (65), 132 (49), 41 (38), 55 (33), 44 (31), 101 (28), 77 (27), 115 (26)
4-hexanolide	334	170 (100), 105 (73), 57 (71), 115 (61), 41 (45), 132 (40), 55 (32), 120 (30), 44 (28), 77 (22)
4-heptanolide	348	105 (100), 57 (96), 184 (95), 132 (75), 41 (73), 55 (65), 115 (58), 44 (49), 43 (46), 77 (43)
4-octanolide	362	57 (100), 105 (95), 132 (89), 198 (80), 41 (78), 55 (75), 115 (58), 77 (50), 44 (49), 43 (40)
4-nonanolide	376	105 (100), 57 (99), 132 (99), 41 (82), 115 (74), 212 (73), 55 (70), 43 (53), 77 (47), 44 (44)
4-decanolide	390	132 (100), 57 (86), 105 (86), 41 (68), 55 (59), 115 (59), 77 (53), 43 (52), 226 (47), 44 (38)
4-undecanolide	404	132 (100), 57 (82), 41 (79), 105 (78), 43 (56), 55 (56), 115 (48), 77 (47), 240 (45), 120 (42)
4-dodecanolide	418	115 (100), 57 (86), 132 (86), 105 (85), 41 (70), 55 (64), 43 (57), 74 (51), 254 (45), 158 (44)
		δ-Lactones
5-hexanolide	334	170 (100), 57 (98), 41 (67), 105 (52), 55 (48), 42 (44), 43 (42), 115 (40), 120 (37), 72 (21)
5-heptanolide	348	184 (100), 57 (88), 105 (78), 132 (73), 41 (62), 115 (60), 55 (55), 120 (47), 74 (43), 44 (41)
5-octanolide	362	198 (100), 57 (80), 105 (73), 55 (67), 120 (54), 115 (49), 41 (48), 74 (35), 43 (30), 132 (28)
5-nonanolide	376	212 (100), 57 (88), 105 (84), 115 (76), 41 (66), 55 (65), 120 (63), 74 (45), 43 (41), 132 (40)
5-decanolide	390	57 (100), 115 (94), 226 (92), 105 (85), 41 (81), 55 (74), 120 (69), 74 (59), 43 (58), 132 (53)
5-undecanolide	404	115 (100), 57 (95), 105 (91), 240 (88), 120 (76), 55 (73), 41 (72), 43 (63), 74 (56), 132 (53)
5-dodecanolide	418	115 (100), 57 (91), 105 (78), 55 (67), 41 (65), 254 (63), 120 (63), 43 (60), 132 (56), 74 (55)

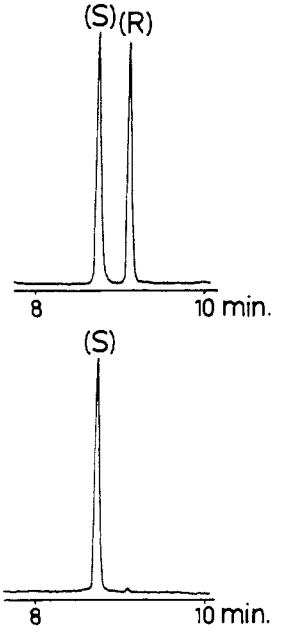

Figure 3. Capillary GC separation of diastereomeric carbamoyl carboxamides derived from (*R,S*)- and (*S*)-4-heptanolide (for conditions see the Experimental Section).

Table II. Separation Factors (α) of Diastereomeric [(*R*)-[(1-Phenylethyl)carbamoyl]oxy] *N*-Butylcarboxamides Derived from γ- and δ-Lactones

lactone	α[a]	*T*,[b] °C
	γ-Lactones	
4-pentanolide	1.123	210
4-hexanolide	1.117	210
4-heptanolide	1.099	210
4-octanolide	1.082	220
4-nonanolide	1.071	220
4-decanolide	1.060	225
4-undecanolide	1.055	225
4-dodecanolide	1.052	225
	δ-Lactones	
5-hexanolide	1.097	210
5-heptanolide	1.090	210
5-octanolide	1.063	220
5-nonanolide	1.052	220
5-decanolide	1.045	220
5-undecanolide	1.036	220
5-dodecanolide	1.032	225

[a] Quotient of retention times of diastereoisomers II and I. [b] Column temperature.

strates the significant impact of the *N*-butylamide moiety on the resolution. For recognition of enantiomers on chiral stationary phases, such formations of N–H-containing derivatives leading to additional functional sites have been widely explored (König, 1987).

The separation factors decrease for the higher lactone homologues; this may be due to the increasing flexibilities of the alkyl side chains. However, the achieved resolutions are still high enough for chirality determinations of the complete spectrum of γ- and δ-lactones, ranging from five to twelve carbon atoms.

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