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Note

Gas chromatographic separation of diastereomeric dicarbamate derivatives of γ- and δ-lactones

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Lactones are an important group of chiral compounds playing significant roles in several foods[1]. In spite of their powerful contributions to overall flavour impressions, the amounts of lactones in natural systems are mostly very low, and sensitive analytical techniques are required to determine their naturally occurring enantiomeric compositions. Recently a major breakthrough has been achieved by direct capillary gas chromatographic (GC) separation of enantiomers of γ-lactones using modified cyclodextrin as a chiral stationary phase[2]. However, the conversion into diastereomeric derivatives followed by separations on non-chiral (commonly available) stationary phases is still a useful and easily applicable alternative for laboratories with standard GC equipment.

The GC separation of diastereomeric ketals obtained by direct reaction with optically pure 2,3-butanediol has been described for δ-lactones[3,4]. In general the formation of diastereomeric derivatives of lactones requires an opening of the ring to obtain intermediates with reactive functional groups. Enantiomers of 4- and 5-hydroxyalkanoic acid esters, derived from γ- and δ-lactones, were separated after conversion into (*R*)-1-phenylethylcarbamates[5]. Opening of the lactone ring to give 4-hydroxyalkanoic acid isopropylesters and subsequent derivatization with (*S*)-O-acetyllactyl chloride[6] was applied to determine the configurations of γ-deca- and γ-dodecalactone in peach[7] and strawberry[8]. The corresponding diastereomeric esters of (*S*)-tetrahydro-5-oxo-2-furancarboxylic acid have been used to investigate chiral δ-lactones[9]. Reactive functional groups can also be obtained by reduction of γ- and δ-lactones to 1,4-and 1,5-diols, respectively. Separations of the corresponding diesters of (*S*)-O-acyllactic acids have been reported[10].

This paper presents the capillary GC separation of diastereomeric dicarbamates obtained by derivatization of 1,4- and 1,5-diols with (*R*)-(+)-1-phenylethyl isocyanate.

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EXPERIMENTAL

Chemicals

(*R*)-(+)-1-Phenylethyl isocyanate (PEIC) and 4-dimethylaminopyridine (DMAP) were obtained from Fluka (Neu-Ulm, F.R.G.). Racemic γ- and δ-lactones were gifts from Haarman & Reimer (Holzminden, F.R.G.) and Firmenich (Geneva, Switzerland). All solvents were redistilled before use.

Derivatization

Reduction of γ- and δ-lactones to 1,4- and 1,5-diols. Lithium aluminium hydride was added to a solution of lactones (in a typical experiment 0.5 μl of an equimolar mixture of C_5–C_{11} γ-lactones) in 0.2 ml dry diethyl ether. After shaking at room temperature for 15 min and addition of 1 *M* HCl (1 ml) the mixture was extracted with chloroform (2 × 20 ml). The chloroform extracts were washed (1 ml $NaHCO_3$, 1 ml water) and dried with sodium sulphate. The solvent was evaporated and the residue redissolved in 200 μl diethyl ether.

Conversion into diastereomeric dicarbamates. After transferring the solution to a screw-capped reaction vial the diethyl ether was removed by using a stream of nitrogen. The residue was dissolved in 25 μl toluene containing 1% DMAP. A 2 μl volume of (*R*)-(+)-PEIC was added and the mixture was kept at 60°C for 24 h. After addition of 250 μl dichloromethane the solution was subjected to GC analysis.

Capillary GC

Capillary GC separations were carried out on a 30 m × 0.32 mm I.D. DB 210 column (film tickness 0.25 μm; J&W Scientific) installed in a Carlo Erba Fractovap Series 2150 gas chromatograph, equipped with a split (1:25) and flame ionization detection (FID). Injector temperature: 230°C. Detector temperature: 275°C. Carrier gas (hydrogen), 0.85 bar; flow-rate, u (225°C) = 55 cm/s. Temperature programme from 220 to 260°C at 1°C/min.

Capillary GC–mass spectrometry (MS)

A Finnigan MAT 4500 series quadrupole gas chromatograph–mass spectrometer coupled with an Incos data system was used. The fused-silica column (described above) was inserted directly into the ion source. Ionization voltage: 70 eV. Ion source temperature: 180°C. Speed: 1 scan/s. Mass range (m/z): 33–500.

RESULTS AND DISCUSSION

Diastereomeric dicarbamates were obtained from chiral γ- and δ-lactones by reduction with lithium aluminium hydride and subsequent derivatization of the 1,4- and 1,5-diols formed, with (*R*)-(+)-1-phenylethyl isocyanate (Fig. 1). The GC separation of diastereoisomers obtained by this procedure from an homologous series of racemic lactones is shown in Fig. 2.

Complete conversion into dicarbamates at moderate temperature was achieved by using DMAP as a catalyst[11]. DMAP has also been employed to obtain carbamate diastereoisomers from the sterically hindered tertiary alcohol linalool[12]. The presence of water and/or traces of acid has to be avoided; they cause the reaction to yield only

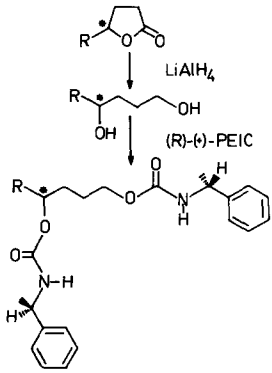

Fig. 1. Conversion of γ-lactones into diastereomeric dicarbamates (R = from $-CH_3$ to $-C_7H_{15}$).

a mixture of mono- and dicarbamates. Quantitative derivatization, however, is necessary to prevent possible discrimination of enantiomers.

The identities of the derivatives were confirmed by GC–MS. Mass spectra of the dicarbamates were mainly characterized by fragmentations of the 1-phenylethylcarbamoyloxy moieties of the molecules. The spectra obtained from an homologous

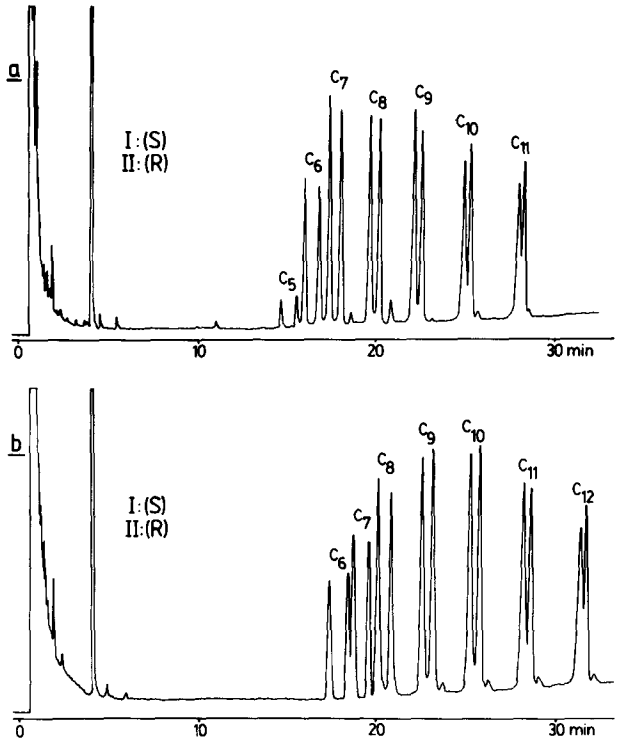

Fig. 2. Gas chromatographic separation of di[(*R*)-1-phenylethyl]carbamates derived from racemic γ-lactones (a) and δ-lactones (b); for conditions see Experimental.

series of lactones were very similar. The ten most intense fragments (m/z, relative intensity, %) of the dicarbamate derived from (*S*)-1,4-octanediol are representative: 105 (100), 120 (60), 132 (47), 164 (21), 106 (32), 166 (30), 150 (29), 69 (25), 77 (18), 147 (16). The spectrum also showed minor but diagnostic fragment ions at M − 15, M − 105, M − 164 and M − 165. Apart from slight differences in the intensities, the mass spectrum of the diastereomeric dicarbamate was identical.

The order of elution was determined by derivatizing optically enriched reference compounds obtained by reduction of oxo precursors using baker's yeast[13,14] and by resolution of racemic lactones by means of chromatography on cellulose triacetate[15]. Within the homologous series of C_5–C_{11} γ- and C_6–C_{12} δ-lactones the dicarbamates derived from (*S*)-lactones are eluted before the derivatives of the corresponding (*R*)-enantiomers.

The derivatives of the higher homologues exhibit only low volatilities. Elution and (partial) resolution of these diastereoisomers without exceeding the upper temperature limit of the column was achieved by using hydrogen as a carrier gas. The separation factors, α, of the diasteromeric pairs separated are listed in Table I. The separation strongly depends on the structures of the lactones. The α values decrease with increasing length of the alkyl side chains of the lactones.

Special attention has to be paid to the fact that the retention times of dicarbamates derived from γ-lactones partly match those of δ-lactones. Starting from chain length C_8, the first dicarbamate eluted derived from δ-lactones is co-eluted with the second of the diastereomeric derivatives obtained from the corresponding γ-lactones. Therefore, in natural systems where both γ- and δ-lactones of the same chain length are present a preseparation, *e.g.*, by means of preparative GC, prior to the derivatization procedure is necessary.

The method described has been applied to determine the naturally occurring enantiomeric compositions of γ- and δ-lactones in mango fruits[16]. Due to the above-mentioned disadvantages (low volatilities and low separation factors of the higher homologues), accurate chirality determinations can be carried out only for lactones up to chain length C_8. Improved derivatization techniques have been worked out and will be published elsewhere[17].

TABLE I

SEPARATION FACTORS (α) OF DIASTEREOMERIC DICARBAMATE PAIRS DERIVED FROM γ- AND δ-LACTONES

Column: DB 210, see Experimental. Column temperature: 240°C (isothermal).

γ-Lactone	α	*δ-Lactone*	α
Valerolactone	1.078	Hexalactone	1.080
Hexalactone	1.069	Heptalactone	1.075
Heptalactone	1.051	Octalactone	1.056
Octalactone	1.039	Nonalactone	1.042
Nonalactone	1.032	Decalactone	1.033
Decalactone	1.023	Undecalactone	1.025
Undecalactone	1.018	Dodecalactone	1.019

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