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ANALYTICAL RESOLUTION OF 4(5)-ALKYLATED $\gamma(\delta)$-LACTONES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON A SILICA-BONDED CHIRAL POLYACRYLAMIDE SORBENT

CHROMATOGRAPHIC CHARACTERIZATION OF A STATIONARY PHASE

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SUMMARY

A silica bonded chiral polyacrylamide phase (Merck Hibar ChiraSpher column, RT 250-4) was used for high-performance liquid chromatographic separation of homologous chiral 4(5)-alkylated $\gamma(\delta)$-lactones (alkyl chain lengths from C_1 to C_8). For all compounds, the order of elution was (*S*)- before (*R*)-enantiomer. Column characteristics, such as the capacity factor, k', number of theoretical plates, N, resolution, R, and H–u curve were evaluated. Furthermore, a correlation between the selectivity, α, and the alkyl chain length of the lactones with the separation mechanism of the system used was attempted.

INTRODUCTION

4(5)-Substituted $\gamma(\delta)$-lactones are widely used as intermediates in the synthesis of natural products and are important, widespread flavour compounds[1,2]. Most of these lactones are chiral compounds and their potential physiological activity, such as, *e.g.*, odour or taste depends on the absolute configuration[3]. Due to the importance of this class of chemicals there have been a number of publications dealing with their chiral analysis. Additionally to gas and liquid chromatographic analysis using achiral phases after derivatization with optically pure reagents[4–8], increasing information about direct enantiomer separation on chiral stationary phases is available. Thus, complexation gas chromatography[9,10] as well as gas chromatography on chiral amide[11] and modified α-cyclodextrin phases[10] have been described. Direct liquid chromatographic separation of lactone enantiomers has been recently achieved using cellulose triacetate[12]. However, this study was carried out with the aim of preparative isolation of lactone enantiomers and, consequently, low pressure and large columns were used, resulting in extremely high retention times.

This paper concerns the direct liquid chromatographic separation of chiral 4(5)-alkylated $\gamma(\delta)$-lactones using a silica-bonded chiral polyacrylamide phase as well as the characterization of the stationary phase.

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EXPERIMENTAL

A Hibar ChiraSpher column (RT 250-4, 250 mm × 4 mm, d_p = 5 μm; Merck, Darmstadt, F.R.G.) was used. With the LC pump 410 (Kontron, London, U.K.), injection valve 7125 (Rheodyne, Cotati, CA, U.S.A.) with a 20-μl sample loop and a variable wavelength UV detector (Knauer, Berlin, F.R.G.), a pressure of 20 bar was found at a flow-rate of 1.2 ml/min at 22°C [eluent composition: *n*-hexane–*tert.*-butyl methyl ether (95:5)]. The wavelength used was 220 nm.

Before use, distilled eluents (*n*-hexane, *tert.*-butyl methyl ether, ethanol, tetrahydrofuran, dioxane) were degassed in an ultrasonic bath. To all eluent compositions, 0.2 ml ethanol/l were added to coat free OH groups of the silica gel in order to avoid undesired adsorptive interactions of the solutes with the sorbent. Furthermore, the samples dissolved more easily in an ethanol-containing solvent.

The solutes were the homologous 4(5)-alkylated γ(δ)-lactones with alkyl chain lengths from C_1 to C_8 (γ-penta- and δ-hexalactone to γ-dodeca- and δ-tridecalactone; Roth, Karlsruhe, F.R.G.).

The determination of the order of elution was performed by multiple injection of the solutes. With approximately 1.5 mg of separated enantiomers obtained by this repeated procedure, the optical rotations were determined. This was achieved using a 241 MC polarimeter and a 1-ml cell (length 10 cm; Perkin-Elmer, Überlingen, F.R.G.) at 546 nm (21°C). By use of knowledge of the correlation between optical rotation and absolute configuration, the order of elution was determined [(*S*)- before (*R*)-enantiomer].

RESULTS AND DISCUSSION

The stationary phase studied consisted of a polar silica, the surface of which had been modified by radical polymerization of optically active acrylamides with (*S*)-phenylalanine ethyl ester residues. For the characterization of such a chiral high-performance liquid chromatographic (HPLC) phase its retention behaviour and selectivity are of particular importance. The quality of the column is determined by analytically non-variable parameters, such as the form and size of the particles, and

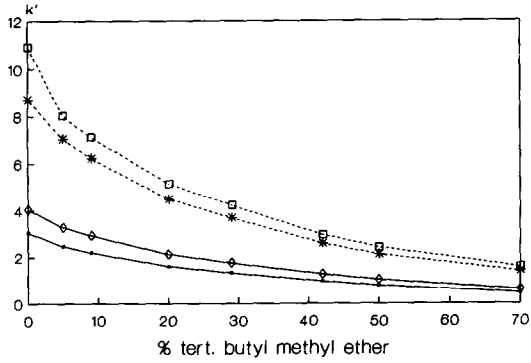

Fig. 1. Dependence of *k'* on the eluent composition. Flow-rate: 1.2 ml/min. ● = (*S*)-γ-Undecalactone; ◇ = (*R*)-γ-undecalactone; * = (*S*)-γ-pentalactone; □ = (*R*)-γ-pentalactone.

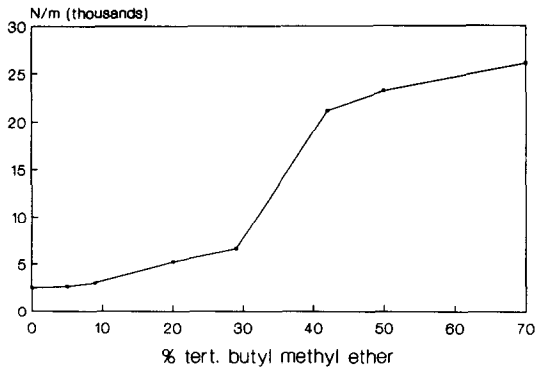

Fig. 2. Dependence of N on the eluent composition. Flow-rate: 1.2 ml/min. Example: (*R*)-γ-undecalactone.

can only be slightly optimized by the composition and flow of the mobile phase as well as the column length.

Since the capacity factor, k', represents the weight ratio of components in the stationary and mobile phases, it depends on the eluent composition. Fig. 1 shows this dependence using an increasing amount of *tert.*-butyl methyl ether (0–70%) in the eluent. For the example of γ-undecalactone enantiomers, amounts of *tert.*-butyl ether up to 10% were necessary to obtain the desired k' values of 2–5[13]. Due to the higher polarity of γ-pentalactone, for this lactone, optimum k' values were found using 10–50% ether in the eluent. Since longer-chain lactones are especially important as flavour components, in the following experiments, based on the k' values determined, 5% ether in the eluent was used.

The dependence of the number of theoretical plates, N, on the eluent composition is outlined in Fig. 2 for the example of (*R*)-γ-undecalactone. Up to 30% ether in the eluent approximately 6500 theoretical plates/m were determined, corresponding to one fourth of the value evaluated for mesitylene (N = 33 813/m), a compound practically not retarded by the column. According to the theory, with increasing eluent polarity N approaches the value for mesitylene. In general, an increase in the

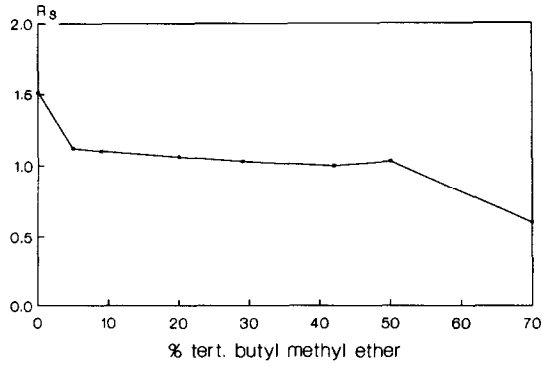

Fig. 3. Dependence of R_s on the eluent composition. Flow-rate: 1.2 ml/min. Example: (*R,S*)-γ-pentalactone.

amount of polar solvent in the mobile phase leads to a shortening of retention times and an improvement in the number of plates. However, parallel to this, k' decreases resulting in a loss of separation capacity. Also in this case, the optimum values were 5–20% ether in the mobile phase (Fig. 2).

The resolution, R_s comprises parameters such as the column selectivity and capacity factors as criteria of analysis time and the theoretical number of plates. The selectivity, α, is independent of the eluent composition. Thus, the development of the curve in Fig. 3 can be discussed by means of k' and N. The strong increase of R_s using eluents containing 0–5% ether can be explained by the great differences of k' found in this region. With eluents containing 5–50% ether, the decrease in capacity factors and increase in the number of plates are approximately equivalent. In this region, a nearly constant resolution was found; the values of R_s = 1.2–1.3 determined are optimal for practical purposes[13]. Exchange of *tert.*-butyl methyl ether by tetrahydrofuran and dioxane resulted (at the same polarity) in a loss in resolution in spite of comparable α values. This phenomenon can be explained by the higher viscosity and, thus, lower diffusion coefficient of the sample as well as a potentially changed swelling behaviour of the polymer film resulting in variation of mass transport in the stationary phase.

In addition to selectivity and retentivity, the efficiency of a chromatographic column is important in order to obtain short analysis times with sufficient resolution. The efficiency of the column studied was checked by measurement of the plate height, H, of retarded peaks as a function of the linear velocity of mobile phase, u. The H–u curve evaluated for (*R*)-γ-decalactone is presented in Fig. 4. The broad minimum observed allows a wide range of application. As a compromise between the least possible plate height, H, and the analysis time, a flow-rate of 0.17 cm/s (= 1.2 ml/min) was selected.

The selectivity of a chromatographic column is determined by the relative retention, α. In order to study the dependence of α on the molecular structure of the sample, the α values for the enantioresolution of homologous γ- and δ-lactones were evaluated as a function of the alkyl chain length, n. As outlined in Fig. 5, an increase in selectivity with increasing chain length was observed, exhibiting a slight minimum for n = 3–5. With $n > 3$, γ-lactones showed higher relative retention than δ-lactones. Similar α values were found in both series of lactones for n = 1–4.

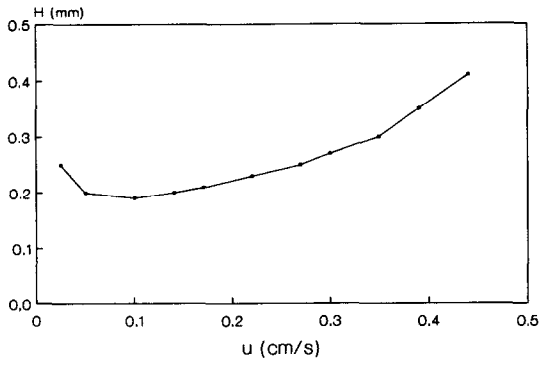

Fig. 4. Plate height, H *vs.* the linear velocity, u. Eluent: 5% *tert.*-butyl methyl ether–*n*-hexane. Flow-rate: 1.2 ml/min. Example: (*R*)-γ-decalactone.

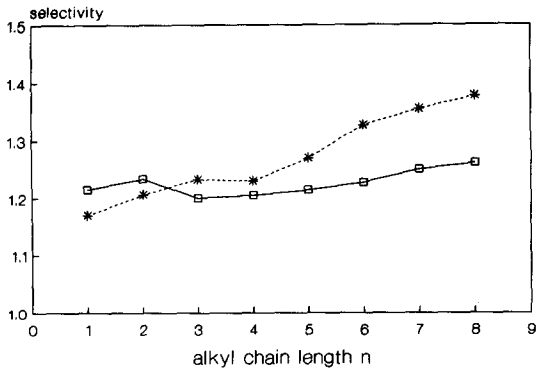

Fig. 5. Selectivity, α, *vs.* the alkyl chain length, *n*. Eluent: 5% *tert.*-butyl methyl ether–*n*-hexane. Flow-rate: 1.2 ml/min. * = γ-lactones; □ = δ-lactones.

These phenomena can be explained by the particular separation mechanism of the column used. The silica-bonded polymer forms a three-dimensional network, in which the molecules of the sample diffuse. Interaction forces, such as dipole–dipole and hydrogen bonding can play an additional role in the interior of the cavities, but in particular the sizes and structures of the sample molecules are essential for the selectivity. The increased selectivity observed for γ-lactones is likely dependent on the half-planar structure of the five-membered ring and, thus, the higher steric differentiation of both enantiomers. With short alkyl chains, this effect is less distinct, resulting in similar α values for the lower homologues of the γ- and δ-lactones. In comparison to cellulose triacetate[12], the phase studied exhibits contrary selectivity.

The limits for detection (signal-to-noise ratio = 2:1) and resolution, (R_s = 0.9) ranged from 0.1 to 50 mg/ml for 20 μl injected. Thus, semipreparative use of the column is also possible (*cf.* Experimental).

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