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SEPARATION OF ENANTIOMERIC AMINES BY ION-PAIR CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic method for the separation of optical isomers (enantiomers) of amines is described. It is based on ion-pair chromatography with a chiral counter ion in a system with an organic mobile phase and an adsorbing stationary phase. The method has been applied to enantiomers of 1-aryloxy-3-isopropylamino-2-propanol derivatives (alprenolol, metoprolol, propranolol) which are completely resolved with (+)-10-camphorsulphonate as the counter ion. Studies of the influence of the counter-ion structure and the mobile phase composition are presented.

INTRODUCTION

Many studies on methods for the separation and determination of enantiomeric organic compounds have been made in recent years. Methods for the transfer of enantiomers to diastereomeric derivatives that can be separated by liquid and gas chromatography have attracted much interest[1,2]. Many investigations have also been focused on the preparation of chiral solid phases for gas or liquid chromatography that can bind the enantiomeric forms with different strengths[3–5]. It has recently been shown that separations can be effected by liquid chromatography with chiral eluents. The investigations have so far been concentrated on the use of ligand exchange in metal complexes[6–8]. Interactions between ions of opposite charge have been used for the purpose of separating enantiomers. Optically active cations have been used as liquid ion exchangers in Craig counter-current extraction for the resolution of sodium *dl*-mandelate, but attempts to use these cations in reversed-phase liquid chromatography have failed[9,10]. Paper chromatographic studies with (+)-10-camphorsulphonic acid or (+)-tartaric acid in the stationary or mobile phase have in some instances resolved a few amines[11–13]. The anion of (+)-tartaric acid has also been used for the resolution of optically active octahedral metal complexes by formation of diastereomeric charged ion pairs in paper electrophoresis and in ion-exchange chromatography[14–16].

In this study, stereoselective association between ions has been used to resolve enantiomeric amines. Diastereomeric ion pairs between the amines and the optically

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active (+)-camphorsulphonic acid are formed and they can have such structural differences that they show different distributions between the phases in the chromatographic system. As the separation is based on the properties of the ion pairs, only such phase systems have been used that favour a high degree of ion-pair formation, *i.e.*, mobile phases of low polarity with solid adsorbents as stationary phases. The investigations have been concentrated on studies of the influence of sample and counter-ion structure and phase composition on selectivity.

EXPERIMENTAL

Apparatus

The detector was a Model 440 UV monitor (Waters Assoc., Milford, MA, U.S.A.) with a 12.5-μl cell, measuring at 254 nm. The pump was an LDC Model 711-26 solvent delivery system. The injector was a Rheodyne Model 7120 with a 20-μl loop. The columns were made of stainless steel with a polished surface, length 100 or 150 mm, I.D. 3.2 mm. The column and injector were thermostated at 25.0 $\pm$ 0.1°C using a water-bath.

Chemical and reagents

LiChrospher SI 1000 (10 μm), LiChrosorb-DIOL (10 μm) and LiChrosorb-DIOL (5 μm) were obtained from E. Merck (Darmstadt, G.F.R.).

Methylene chloride (p.a. grade, Merck) was freed from water before use by passage through a molecular sieve column (4 Å). The water content was less than 0.001%. (+)-10-Camphorsulphonic acid, (+)-3-bromo-10-camphorsulphonic acid and (+)-3-bromo-8-camphorsulphonic acid were obtained from Merck. Racemic alprenolol chloride, (+)-alprenolol chloride, (—)-alprenolol tartrate and racemic metoprolol tartrate were supplied by Hässle (Mölndal, Sweden), racemic propranolol chloride by ICI (Macclesfield, Great Britain) and racemic oxprenolol chloride by Ciba-Geigy (Basle, Switzerland). All other substances and solvents were of analytical or reagent grade and were used without further purification.

Chromatographic technique

The columns were packed with a slurry technique using chloroform as the slurry medium. They were washed with methanol, methylene chloride and finally *n*-hexane before use. The mobile phase containing the counter ion in acidic form was recirculated through the column and constant retention times were reached after about 2 days.

The samples were introduced as bases or salts dissolved in the mobile phase.

RESULTS AND DISCUSSION

The proposed method for the separation of enantiomeric amines as ion pairs with a chiral counter ion is based on the assumption that diastereomeric ion pairs can have different distributions between an organic mobile phase and a stationary adsorbent. However, differences cannot be expected if the ion pair components are bound only by electrostatic attraction between the charged groups and it is, as a rule, assumed that interaction at three points in the vicinity of the chiral carbon atom is

necessary to give chiral ion pairs with different mobile phase solvation or stationary phase adsorption (*cf.* Dalgliesh's "three-point" rule[17]).

The main group of enantiomeric amines used in this study contained a so-called β-chain (Fig. 1). The chain carries, in addition to the secondary amino group, a hydroxyl group at the chiral carbon in position 2. (+)-10-Camphorsulphonate (Fig. 2) was used as the counter ion in the main part of the studies. It is an aprotic anion with a rigid structure with an oxo group in position 2.

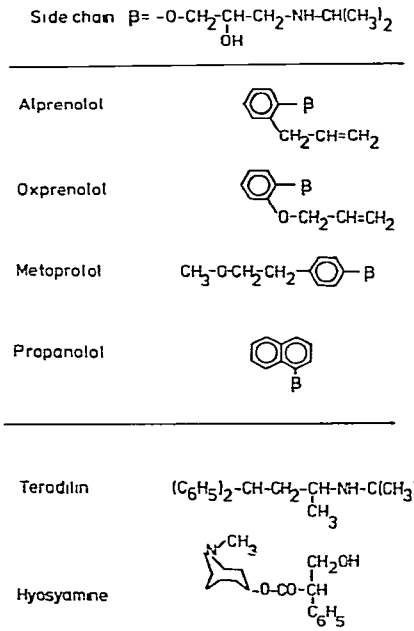

Fig. 1. Sample structures.

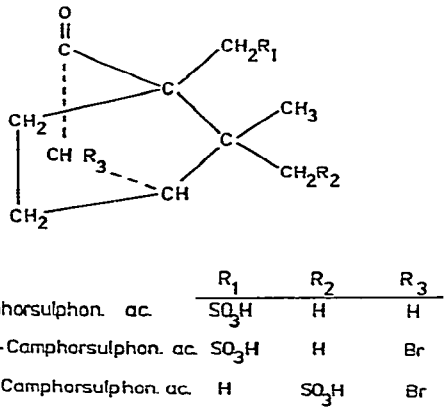

Fig. 2. Counter-ion structures.

Ion pairs between these components can be bound not only by electrostatic attraction but also by hydrogen bonding between the hydroxyl and the oxo group as well as by hydrophobic interaction between the ring systems. Three interaction points, *i.e.*, the basic prerequisites for separation, seem to exist.

Influence of phase composition

The separations were usually performed with LiChrosorb-DIOL as stationary phase and methylene chloride + 1-pentanol as mobile phase. Test runs with more hydrophobic phases (Nucleosil CN and LiChrosorb RP-2) gave highly asymmetric peaks. The more hydrophilic LiChrospher SI 1000 gave too high a retention.

It is of vital importance for the separation selectivity to keep the content of polar components in the mobile phase as low as possible. Water, even in very low concentrations, seems to have a very adverse influence on the separation and no separation of the enantiomers could be obtained if the chromatographic system contained a stationary aqueous phase. The influence of other less strongly hydrogen bonding

agents is illustrated in Table I for alprenolol. Lipophilic alcohols of different structures (1-pentanol, and isopropanol) seem to give about the same selectivity. Hydrogen-donating agents such as acetonitrile, tetrahydrofuran and ethyl acetate might give slightly higher separation factors, when used at the same concentration as the alcohols. However, the peak asymmetry is significantly greater, which makes them less suitable than, *e.g.*, 1-pentanol. An increase in the 1-pentanol content from 0.5% to 5% decreased the separation factors from 1.08 to 1.02.

TABLE I

INFLUENCE OF POLAR COMPONENTS IN THE MOBILE PHASE

Mobile phase: (+)-10-camphorsulphonate, $2.1 \cdot 10^{-3}$ *M* in methylene chloride–polar solvent (flow-rate, 3.2 mm/sec). Sample: alprenolol (+ and − forms).

Polar solvent	*Content (%)*	*k' (+)*	*k' (−)*	*Separation factor (α)*	*H (mm)*	*Asf**
Pentanol	0.5	20.3	22.0	1.08	0.15	1.5
	1	10.8	11.5	1.06	0.15	1.1
	5	2.02	2.07	1.02	0.15	1.1
Isopropanol	0.5	13.5	14.5	1.07	0.18	2.6
Acetonitrile	1	25.1	27.3	1.09	0.20	2.2
Tetrahydrofuran	1	11.9	13.1	1.10	—	3.7
Ethyl acetate	1	29.0	31.8	1.10	—	3.6

* Asf = back part of the peak/front part of the peak.

The negative influence of the hydrogen-bonding agents is probably due to their interaction with hydrogen-bonding groups of the ion-pair components, which decreases the bonding strength within the ion pair.

All of the chromatographic systems had a fairly low separation efficiency. The 1-pentanol-containing systems, which were the most efficient, showed a reduced plate height of 12–15 even though testing of the column packing before conditioning with camphorsulphonate gave a reduced plate height of 4.

Molecular structure and chiral selectivity

The influence of the sample structure on the separation factor in a system with (+)-10-camphorsulphonate as counter ion is illustrated in Table II. The results are in agreement with the assumptions regarding the interactions between the ion-pair

TABLE II

SEPARATION OF ENANTIOMERS

Mobile phase: (+)-10-camphorsulphonate, $2.2 \cdot 10^{-3}$ *M* in methylene chloride–1-pentanol (199:1).

Substance	*k' (+)*	*k' (−)*	*Separation factor*
Alprenolol	24.9	27.4	1.10
Metropolol	34.9	38.7	1.11
Oxprenolol	15.0	15.0	1.00
Propranolol	40.9	46.0	1.12
Terodiline	4.9	4.9	1.00
Hyoscyamine	7.3	7.3	1.00

components. Fairly high separation factors are obtained for most of the 1-aryloxy-3-isopropylamine-2-propanol derivatives and complete resolution of the enantiomers is possible on a 400-mm column.

The enantiomers of oxprenolol are not separated, probably owing to the presence of a hydrogen-accepting 2-allyloxy group in such a position in the ring that internal hydrogen bonding with the hydroxyl in the side-chain is possible. Terodilin, which lacks the hydroxyl group, and hyoscyamine, which has the hydroxyl group too distant from the amino group, show no separation into enantiomers.

A further illustration of the importance of a good fit between the binding groups in the ion-pair components is given by a study of the separation of the enantiomers of alprenolol with three different camphorsulphonates (Fig. 2) as counter ions. Introduction of a 3-bromo substituent in 10-camphorsulphonate brings about a complete loss of stereoselectivity, possibly owing to steric repulsion by the bromo atom preventing a close three-point contact between the ion-pair components. A slight stereoselectivity ($\alpha = 1.02$) remains, however, when the sulphonate group is situated in position 8, *i.e.*, more distant from the oxo group.

Retention principles

The retention decreases with increasing hydrophobicity of the ion-pair components, which shows that the solid adsorbent, as expected, is the more polar phase. An increase in the concentration of camphorsulphonic acid in the mobile phase also gives rise to a decrease in the retention. This might indicate some kind of ion-exchange mechanism with camphorsulphonic acid acting as stationary phase, but other retention models are also possible.

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REFERENCES

1 D. R. Knapp, *Handbook of Analytical Derivatization Reactions*, Wiley, New York, 1979, p. 405.
2 E. Gil-Av and D. Nurok, *Advan. Chromatogr.*, 10 (1974) 99.
3 R. Audebert, *J. Liquid Chromatogr.*, 2 (1979) 1063.
4 W. H. Pirkle, D. W. House and J. M. Finn, *J. Chromatogr.*, 192 (1980) 143.
5 G. Blaschke, *Angew. Chem., Int. Ed. Engl.*, 19 (1980) 13.
6 J. N. LePage, W. Lindner, G. Davies, D. E. Seitz and B. L. Karger, *Anal. Chem.*, 51 (1979) 433.
7 W. Lindner, J. N. LePage, G. Davies, D. E. Seitz and B. L. Karger, *J. Chromatogr.*, 185 (1979) 323.
8 P. E. Hare and E. Gil-Av, *Science*, 204 (1979) 1226.
9 S. J. Romano, K. H. Wells, H. L. Rothbart and W. Rieman, III, *Talanta*, 16 (1969) 581.
10 W. Rieman III, N. Luque and J. Jimenez, *Separ. Sci.*, 8 (1973) 193.
11 G. B. Bonino and V. Carassiti, *Nature (London)*, 167 (1951) 569.
12 S. Berlingozzi, G. Serchi and G. Adembri, *Sperimentale, Sez. Chim. Biol.*, 2 (1951) 89.
13 S. Berlingozzi, G. Adembri and G. Bucci, *Gazz. Chim. Ital.*, 84 (1954) 393.
14 H. Yoneda and T. Miura, *Bull. Chem. Soc. Jap.*, 42 (1970) 574.
15 L. Ossicini and C. Celli, *J. Chromatogr.*, 115 (1975) 655.
16 H. Yoneda, *J. Liquid Chromatogr.*, 2 (1979) 1157.
17 C. E. Dalgliesh, *J. Chem. Soc.*, (1952) 3940.