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LIQUID CHROMATOGRAPHIC SEPARATION OF ENANTIOMERIC ALKANOLAMINES VIA DIASTEREOMERIC TARTARIC ACID MONOESTERS

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SUMMARY

A new resolution method for the analytical and preparative separation of enantiomeric alkanolamines into their antipodes is presented. It involves formation of the monoesters of the alkanolamines with optically pure and symmetrically O,O-disubstituted (*R,R*)- or (*S,S*)-tartaric acids. The derivatization reactions are carried out in aprotic media by reaction with the tartaric acid anhydrides. The amine functions are ionically blocked via ion-pair formation with strong acids, *e.g.* trichloroacetic acid. The resulting diastereomeric monoesters are easily separable into their antipodes by reversed-phase liquid chromatographic technique. Relative retention factors, α, up to 4 can be obtained, depending on the structural parameters of the alkanolamines and reagents, as well as on the mobile phase conditions (pH). The monoesters are zwitterions, possibly capable of forming intramolecular ion pairs via a ring structure favoured for ethanolamines.

INTRODUCTION

Liquid chromatography (LC) has been widely used for the separation of diverse enantiomeric compounds into their optical antipodes. Depending on the chemical structure of the solutes, various enantioseparation techniques can be used for chromatographic resolution; recent reviews on this general subject give evidence of the importance of this fast-growing field[1–3].

In principle, two avenues are open to this goal, the so-called direct and indirect enantioseparation methods, whereby all enantioselective techniques not dealing with derivatization reactions with optically active reagents can be summarized as being direct. Diastereomeric derivatives, separable as such by conventional LC systems, are formed via the derivatization procedure.

Focusing on the indirect technique, several requirements should be kept in mind for more general use. (a) The solute molecule must contain at least one —but not two similar— functional groups for a derivatization, *e.g.* amino, hydroxy, or

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carboxyl groups. (b) The optically active reagent (chiral selector, SE) must have extremely high purity, because this has a direct influence on the accuracy of the maximum detectable optical purity of the chiral solutes (selectands, SA). (c) The reaction conditions must be mild so that virtually no racemization of the chiral centres of the selector and selectand occurs. (d) For the analytical determination of the enantiomeric excess (ee), *e.g.* (*R*) besides the (*S*) antipode of a chiral solute (SA), the derivatization reaction must be quantitative, because the constants (k_1 and k_2) of the reactions

$$(R)\text{-SE} + \left\{ \begin{matrix} (R)\text{-SA} \\ (S)\text{-SA} \end{matrix} \right\} \begin{matrix} \xrightarrow{k_1} (R)\text{-SE–}(R)\text{-SA} \\ \xrightarrow{k_2} (R)\text{-SE–}(S)\text{-SA} \end{matrix}$$

are mostly unequal; incompleteness would lead to a miscalculation of the real value of the ee of one antipode. (e) Most important is the steric conformation of the chiral centre(s) and the distance(s) to the reactive functional group(s) in the SE and SA molecules; favourable and unfavourable spatial arrangements of the chiral substituents with respect to each other in the resulting diastereomeric (*R*)-SE–(*R*)-SA, *e.g.* (*R*)-SE–(*S*)-SA, derivatives reflect their different lipophilicity or polarity. Therefore, these arrangements have a direct influence on adequate resolvability (resolution) by diverse chromatographic systems. (f) Additionally, for analytical purposes, SE reagents should have a chromophore or fluorophore to enhance the detectability of the derivatives. (g) For preparative applications the SE reagent should be relatively inexpensive and easily synthesized and, possibly, recycled. (h) In connection with (g), in order to obtain the separated optically pure SA parent molecules, their diastereomeric derivatives should be capable of being cleaved under mild conditions without racemization.

Based on the foregoing, there are no chiral SE reagents available that would fulfill all demands, especially (g), for resolving solute molecules with many different structures and capable of forming derivatives. However, in consideration of many limitations inherent in the indirect and direct enantio-separation techniques, the search for new efficient chiral SE reagents seems worthwhile.

Focusing in the following on the enantioseparation of alkanolamines by LC techniques, both direct and indirect methods have been partially successful, but they are strongly dependent on structural elements of the SE and SA molecules. Thus Petterson and Schill[4] have demonstrated very elegantly the usefulness of (+)-10-camphorsulphonic acid as a chiral ion-pair reagent for the direct resolution of some amino alcohols of the β-blocker series (*e.g.* propranolol, metoprolol). Based on the results of Prelog *et al.*[5], Petterson and Stuurman[6] resolved ephedrine analogues by ion-pair formation of the chiral amines by using a non-chiral lipophilic counter-ion. These ion-pairs could be stereoselectively extracted and/or partitioned into a chiral stationary phase created by adsorption of a lipophilic chiral mobile phase additive (*R,R*-di-*n*-butyl tartrate) on a reversed-phase system. Chiral biopolymers can also function as chiral selectors (SE) for resolving some amino alcohols[7]. The so-called "Pirkle phases", based on $\pi\pi$- and hydrogen-bonding interactions, are widely used valuable techniques. Thus, some alkanolamines could be resolved on such systems,

either directly[8] or after non-chiral derivatization[9,10].

In addition to the direct resolution of selected amino alcohols, some indirect methods have also been reported.

For the determination of (*R*) in the presence of (*S*) alkanolamines with β-adrenergic activity, the chiral reagents listed in Table I have been used. They all form stable N-derivatives with the alkanolamines, in which the hydroxy group remains unaffected. However, the latter is bonded only to the chiral centre. As is well known, with increasing distance between the chiral centres in diastereomeric derivatives, the physicochemical properties of the two optical antipodes become more similar, and this results in lower α values (quotient of the relative retentions of the pair of optical antipodes) in chromatographic systems[2]. As a result, α values between 1.05 and 1.30 —typically around 1.20— were obtained, which are usually sufficient for baseline separations.

Nevertheless, based on the requirements discussed earlier, the search for new types of chiral reagent and/or derivatization procedure seems meaningful, especially if one also concentrates on (semi)preparative resolution of the diastereoisomers and recovery of the parent enantiomers. (*R,R*)- and (*S,S*)-tartaric acid, a natural and readily available optically pure chiral source, should fulfill the most important requirements (a) to (g) of a chiral reagent. It therefore served as a starting material for the chiral reagents summarized in Table II, and subsequently examined with respect to their enhancement of "stereospecificity" and consequently "chromatographic stereoselectivity". This term includes all phenomena affecting the relative retention of a pair of diastereomeric optical antipodes in diverse chromatographic systems.

EXPERIMENTAL

Apparatus and chromatography

The HPLC system consisted of the following components: pump Model 410 (Kontron); detector Model 440 λ 254 nm (Waters); injector Model 7120 with 20-μl loop (Rheodyne); recorder Model 56 (Perkin-Elmer). The columns used were of stainless steel; the analytical column, 250 × 4 mm I.D., was packed with Polygosil RP-18, 7 μm (Machery-Nagel); the precolumn, 50 × 4.6 mm I.D., was inserted before the injector and was packed with LiChrosorb RP-18, 25 μm (Merck). The silica gel

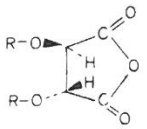
TABLE I

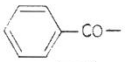
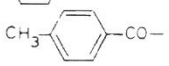
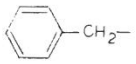
CHIRAL REAGENTS FOR RESOLVING ENANTIOMERIC AMINO ALCOHOLS VIA DERIVATIZATION

Reagents	*Solutes (SA)*	*Ref.*
t-BOC-L-ala anhydride	Propranolol	11
t-BOC-L-leu anhydride	Alprenolol Metoprolol	12
N-Trifluoroacetyl-*S*-(−)-prolylchloride	Propranolol	13
2,3,4-Tri-O-acetyl-α-D-arabinopyranosyl isothiocyanate 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate	Eleven β-blockers	14
S(−)-1-Phenylethyl isocyanate	Propranolol	15
R(−)-1-(1-Naphthyl)ethyl isocyanate	Oxprenolol	16, 17
	Propranolol	18

TABLE II

CHIRAL REAGENTS BASED ON (*R,R*)-TARTARIC ACID ANHYDRIDE



R	*No.*	*Reagents*	*Abbreviation*	*Ref.*
CH_3CO–	1	(*R,R*)-O,O-diacetyl tartaric acid anhydride	DATAAN	19
	2	(*R,R*)-O,O-dibenzoyl tartaric acid anhydride	DBTAAN	20
	3	(*R,R*)-O,O-di-*p*-toluoyl tartaric acid anhydride	DTTAAN	21
CH_3	4	(*R,R*)-O,O-dimethyl tartaric acid anhydride	DMTAAN	22
C_2H_5	5	(*R,R*)-O,O-diethyl tartaric acid anhydride	DEOSAAN	23
	6	(*R,R*)-O,O-dibenzyl tartaric acid anhydride	DBOSAAN	24

columns (Lobar) for the preparative separation of the alkanol derivatives were from Merck. The compositions of the various mobile phases are shown in the corresponding figures and tables.

Chemicals and reagents

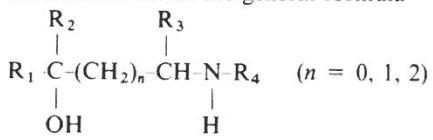
The following solvents for chromatography and synthetic work, including derivatization, were p.a. grade from Merck: *R,R*-(+)-tartaric acid, benzoyl chloride, acetyl chloride, acetic anhydride, toluoyl chloride, sodium hydride, benzyl bromide, methyl iodide, ethyl bromide, acetic acid, methanol, acetone, 2-propanol. The following compounds were generously donated: (±) and (+)- and (−)-propranolol, (±)-atenolol (ICI, U.K.); (±) and (+)- and (−)-pindolol (Sandoz, Switzerland); (±) and (−)-metoprolol and its analogues, (±)-alprenolol (Hässle, Sweden); (±)-oxprenolol (Ciba, Switzerland); (±)-celiprolol (Chemie Linz, Austria); (±)-acebutolol (Bayer, F.R.G.); (±)-methypranolol (Boeringer-Mannheim, F.R.G.); (±)-bupranolol (Bender, Austria); (±)-timolol (MSD, U.S.A.); (±)-bunitrolol, (±)-carazolol, (±)-labetolol, and (±)-nifenalol were extracted from pharmaceutical formulations.

The chiral reagents 1–6 (Table II) were synthesized according to general procedures described in the literature.

RESULTS AND DISCUSSION

Derivatization procedures

Alkanolamines of the general formula



(n = 0, 1, 2)

have two functional groups suitable for derivatization, the hydroxyl and the amino groups. As pointed out earlier (Table I), the amine has always been derivatized with diverse chiral reagents to form amides or urethanes, compounds that are chemically relatively stable and not easy to cleave. Esters, on the other hand, are known to be solvolysed under mild conditions. In order to obtain the ester derivatives exclusively of alkanolamines, the amine function must be protected. In addition to the well-established methods of protein chemistry for forming covalent derivatives, it should be possible to form stable ion-pairs with strong acids, *e.g.*, with sulphonic acids or trichloroacetic acid, which dissociate very little in aprotic solvents. Under those conditions, only the alcohol function will react with the O,O-disubstituted tartaric acid anhydrides to form diastereomeric monoesters (see Fig. 1 and Table II).

The mixture of diastereomeric products can be resolved into its optical antipodes by diverse separation techniques, *e.g.* normal- or reversed-phase chromatography or thin-layer chromatography (but also extraction or crystallization methods), since the relative retention values (based on differences in lipophilicity and polarity) of the pairs of antipodes, depending on structural elements of the reaction partners, can be exceptionally high (see Tables III and IV).

From the resolved and optically pure antipodes of the monoesters, the parent optically pure alkanolamines are easily recovered by solvolysis in protic solvents. The overall yield is usually *ca.* 70% or higher. A detailed description of experimental conditions for some examples will be published later[25].

Preparative scale. The (±)-alkanolamine base, 10 mM, (*e.g.* (±)-propranolol) and 15 mM trichloroacetic acid are dissolved in 300 ml of 1,2-dichloroethane or another aprotic solvent and *ca.* 10 ml of an azeotropic mixture of dichloroethane and any water present is distilled off. To the cooled solution, 20 mM of a chiral

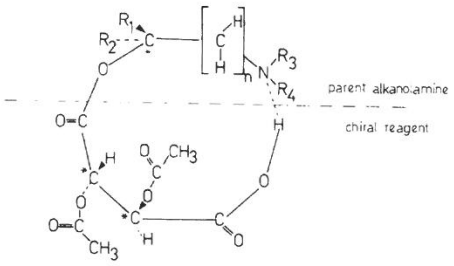


Fig. 1. Model of an intramolecular ring structure of O,O-disubstituted tartaric acid monoesters of alkanolamines.

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