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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

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

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 ππ- 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

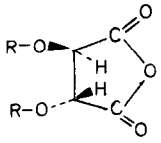
TABLE I

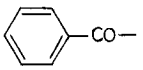
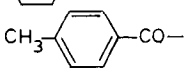
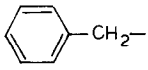
CHIRAL REAGENTS FOR RESOLVING ENANTIOMERIC AMINO ALCOHOLS VIA DERIVATIZATION

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 Propranolol	16, 17 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

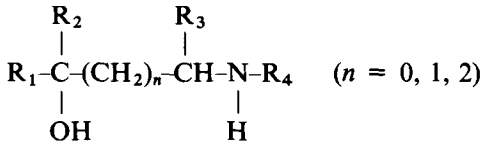
The following solvents for chromatography and synthetic work, including derivatization, were p.a. grade from Merck: *R,R*-(+)-tartaric acid, benzoyl chloride, acetyl chloride, acetic anhydride, toluoyl chloride, sodium hydride, benzyl bromide, methyl iodide, ethyl bromide, acetic acid, methanol, acetone, 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 m*M*, (*e.g.* (±)-propranolol) and 15 m*M* 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 m*M* of a chiral

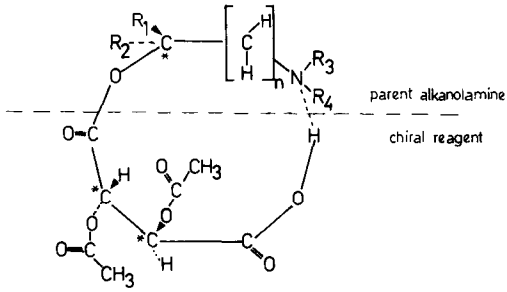


Fig. 1. Model of an intramolecular ring structure of O,O-disubstituted tartaric acid monoesters of alkanolamines.

tartaric acid anhydride (*e.g.* DATAAN, see Table II) is added at once. The solution is brought to *ca.* 50°C for *ca.* 10 min to 4 h to complete the ester formation. The reaction time depends on the steric accessibility of the hydroxy function. After cooling, 200 ml of diluted aqueous ammonia is added to the organic solvent and rigorously shaken or mixed. The excess of reagent and the trichloroacetic acid dissolve in the aqueous phase. The organic layer is separated and the washing procedure is repeated with pure water. The organic layer, containing the mixture of the diastereomeric tartaric acid monoesters, is evaporated to dryness. Depending on the chiral reagent and the alkanolamine (β-blocker), the residue can be redissolved in organic solvents (*e.g.* acetone for propranolol esters) and crystallized. The yield is usually *ca.* 80% or higher.

Analytical scale. In a conical tube, several nanograms to milligrams of free alkanolamines are dissolved in 1 ml or less of a dry, aprotic solvent (*e.g.* dichloroethane, tetrahydrofuran, acetone), and an excess of trichloroacetic acid and at least a three-fold excess of a chiral reagent (see Table II) are added. The reaction vessel is sealed with an appropriate cap, and the reaction mixture is placed in an oven at *ca.* 50°C and allowed to react completely for several hours. The reaction time depends, in addition to structural parameters, on the dilution of the samples. Depending on the compatibility of the reaction solvent with the mobile phase of the respective chromatographic system used, the reaction solvent may or may not be evaporated, and the residue is redissolved in, for example, methanol for direct injection of the reaction mixture into a reversed-phase HPLC column.

The objective of the described derivatization procedure on an analytical scale is twofold. First, to determine the optical purity of particular alkanolamines, and second, to study structural influences of both the chiral SE and SA molecules, also with regard to conformational analysis, based on retention characteristics. First of all, focusing on reversed-phase HPLC systems with normal RP-8 or RP-18 columns, the separation factors of pairs of corresponding diastereomeric compounds are predominantly influenced by differences in lipophilicity and polarity. Dealing with ionizable solutes, their ionization stages, controlled via the mobile phase conditions (buffer pH), will have additional direct influence on the overall chromatographic behaviour.

As is evident from the schematic structural formula of the diastereomeric tartaric acid monoesters of alkanolamines (Fig. 1), an intramolecular zwitterionic ring structure could be invoked. Evidence for this hypothesis is given by the exceptionally high α values obtained, depending on the pH of the mobile phase, (Fig. 2). Going from low to high pH, the α values go through a maximum at pH *ca.* 5.0, and some anomalies of the k' and α curves are observable. This indicates some sensitive conformational phenomena, reflecting differences in the overall lipophilicity of the diastereomeric pairs of antipodes. A small contribution to the uneven shape of the curves might also be due to some experimental factors, since differences in total ionic strength and the concentration of organic modifier in the mobile phase, as well as the temperature, will have an influence on the total retention characteristics. A general trend of the curves of the k' values and the resulting α values can be realized, but it will be expressed differently, depending on structural influences of the chiral SE and SA molecules. The shape of the k' curves also indicates that we are dealing with zwitterionic compounds with an "isoelectric zone" at pH *ca.* 5–6.5. The exact

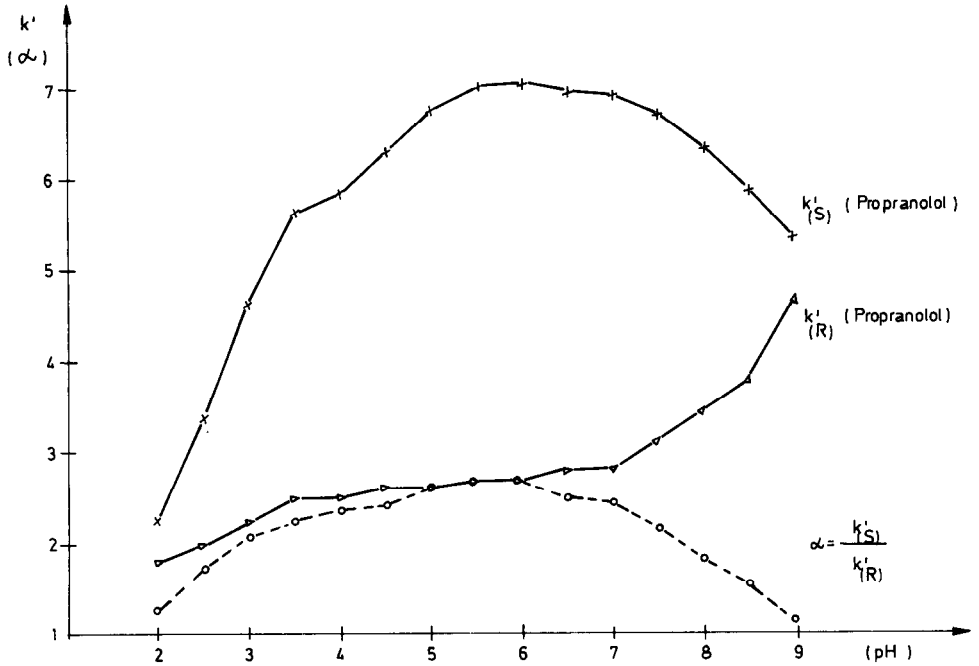


Fig. 2. Relative retention values of the zwitterionic tartaric acid monoester as a function of pH of the mobile phase. Conditions: column, 250 × 4 mm I.D., RP-18, 7 μm; mobile phases, 0.2 *M* phosphoric acid [adjusted to actual pH with ammonia–methanol (1:1)].

pK_a values of the ionizable groups have not been measured, and the isoelectric point is still unknown.

Under these mobile phase conditions, the maximum of the α values is reached at pH *ca.* 5–6.5 (the actual pH is measured and adjusted in the methanolic mobile phase). Small differences of the maxima of the *k'* values and α curves cannot be interpreted yet, but might be caused by more pronounced differences in the lipophilicity and polarity of the solvolysed total molecule complexes rather than by dissociation constants only. The elution order of the diastereomeric derivatives in the different chromatographic systems was confirmed by comparison with derivatives of optically pure (enriched) (*R*)- and (*S*)-alkanolamines, respectively. It is strongly related to the conformation of the molecules. Therefore, it seems safe to assume that the elution order remains the same. Thus, the peak notations of alkanolamines with similar structure, for which optically pure antipodes were not available, seems correct. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) studies have shown that we are dealing with the compounds and structures shown in Fig. 1.

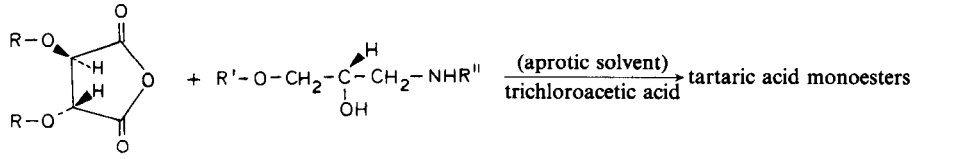
Additionally, and not surprisingly, the substituents on the two oxygens of tartaric acid influence the total "diastereoselectivity" of the derivatized alkanolamines, but can never be seen isolated from the alkanolamine structure.

From Table III it is clear that the bulkiness of the substituents in the reagent molecule is a predominant factor. If *R* is changed from an acetyl to a *p*-toluoyl group in the chiral reagent, the α values increase from 1.6 to 3.4, together with a drastic increase of the *k'* values. For other alkanolamines the same trend will be observed,

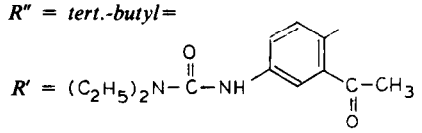
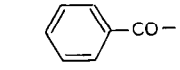
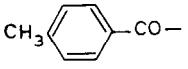
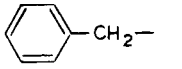
TABLE III

DIASTEREOSELECTIVITY OF CHIRAL REAGENTS, BASED ON O,O-DERIVATIVES OF (*R,R*)-TARTARIC ACID ANHYDRIDE

Chromatographic conditions: column, 250 × 4 mm I.D., RP-18, 7 μm; mobile phase, 0.2 *M* ammonium phosphate buffer (pH 6.0)–methanol (50:50).



(*R,R*)-reagent (parent (*R,S*)-alkanolamine) β-blocker (*R,R*)-reagent–(*R*)-aminoalcohol and (*R,R*)-reagent–(*S*)-aminoalcohol

Reagents		*β-Blocker celiprolol* *R''* = *tert.-butyl*= *R'* = 		
R	*Abbreviation*	$k'_{(R)}$*	$k'_{(S)}$*	$\alpha = \frac{k'_S}{k'_R}$
CH_3CO-	DATAAN	1.30	2.10	1.61
	DBTAAN	4.29	12.29	2.86
	DTTAAN	38.5	134.0	3.48
CH_3-	DMOSAAN	1.35	1.80	1.33
C_2H_5-	DEOSAAN	2.10	3.62	1.73
	DBOSAAN	34.0	51.2	1.51

* The conformation of the parent alkanolamines is indicated by (*R*) and (*S*). This designation is also used in Tables IV–VI and Figs. 2 and 3.

but the magnitudes can be quite different. Also interesting is the fact that, if one exchanges the phosphoric acid in the mobile phase for acetic acid, the resulting α values can be even higher; *e.g.* at a comparable mobile phase composition, the α values for metoprolol are 2.00 and 2.50, respectively. The reason for this cannot be attributed to simple pH effects. The latter mobile phase conditions and the derivatization procedure for the analytical scale lead to the chromatographic parameters and results listed in Tables IV and V for (*R,R*)-O,O-di-acetyl and di-benzoyl tartaric acid monoesters of several β-adrenergic alkanolamines. The cited β-blockers differ chemically mainly in the structure of the phenoxy group. The amine function is substituted by either an isopropyl or a *tert.*-butyl group. The structural dependency

TABLE IV

HPLC SEPARATION OF (*R,S*)-ALKANOLAMINES, O-DERIVATIZED WITH O,O-DIACETYL (*R,R*)-TARTARIC ACID ANHYDRIDE

Conditions: HPLC column, 250 × 4.6 mm I.D., packed with Spherisorb RP-18, 5 μm; mobile phase, 2% acetic acid in water (adjusted with ammonia to pH 3.7)–methanol (50:50); flow-rate, 1.5 ml/min; detection, UV 254 nm.

Alkanolamines (R,S)	$k'_{(R)}$	$k'_{(S)}$	$\alpha = \frac{k'_{(S)}}{k'_{(R)}}$
Propranolol	2.50	6.0	2.40
Oxprenolol	0.70	1.60	2.30
Metoprolol	0.67	1.67	2.50
Celiprolol	0.65	1.50	2.30
Alprenolol	0.70	1.60	2.30
Carazolol	0.70	1.50	2.14

of the chromatographic stereoselectivity of tartaric acid derivatives can also be demonstrated by varying the chain length of the methylene spacer groups between the alcohol and the amine function. Thus, the postulated intramolecular ring size (see Fig. 1) will also change. As a result, the "stereoselectivity" of such derivatives drops quite significantly to α values that are approximately in the range of comparable derivatized enantiomeric alcohols (α *ca.* 1.20). This observation is not a proof for the postulated ten-membered ring structure, but an indication of a sterically favoured intramolecular folding. The influence of the size and structure of the amine

TABLE V

HPLC SEPARATION OF (*R,S*)-ALKANOLAMINES, O-DERIVATIZED WITH O,O-DIBENZOYL TARTARIC ACID ANHYDRIDE

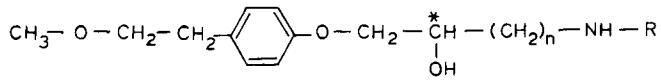
Conditions: HPLC column, 250 × 4.6 mm I.D., packed with Polygosil RP-18, 7 μm; mobile phase, 2% acetic acid in water (adjusted with ammonia to pH 3.7)–methanol (35:65); flow-rate, 1.5 ml/min; detection, UV 254 nm.

Alkanolamines	$k'_{(R)}$	$k'_{(S)}$	$\alpha = \frac{k'_{(S)}}{k'_{(R)}}$
Propranolol	4.00	11.0	2.75
Bupranolol	3.25	10.9	3.35
Oxprenolol	2.40	6.8	2.83
Carazolol	2.38	6.38	2.68
Alprenolol	2.38	6.38	2.68
Timolol	2.33	6.22	2.67
Methypranolol	2.13	8.38	3.93
Pindolol	1.88	4.88	2.59
Metoprolol	1.38	4.63	3.35
Labetolol	1.28	6.39	4.99
Celiprolol	1.13	3.0	2.65
Acebutolol	0.88	2.75	3.13
Bunitolol	0.88	3.13	3.55
Nifenalol	0.63	3.0	4.80
Atenolol	0.35	1.10	3.11

TABLE VI

RETENTION AND DIASTEREOSELECTIVITY OF DATAAN-DERIVATIZED ALKANOLAMINE ANALOGUES

For derivatization and chromatographic conditions, see Experimental.



parent (*R,S*)-alkanolamines

n	*R*	$k'_{(R)}$	$k'_{(S)}$	$\alpha = \frac{k'_{(S)}}{k'_{(R)}}$
3	Isopropyl	1.00	1.28	1.28
2	Isopropyl	0.87	1.04	1.19
1	Isopropyl	0.85	1.85	2.18
1	$C(CH_3)_3$	1.10	2.07	1.88
1	n-C_3H_7	0.76	1.71	2.25
1	C_2H_5	0.59	1.28	2.18
1	H	0.45	0.77	1.31

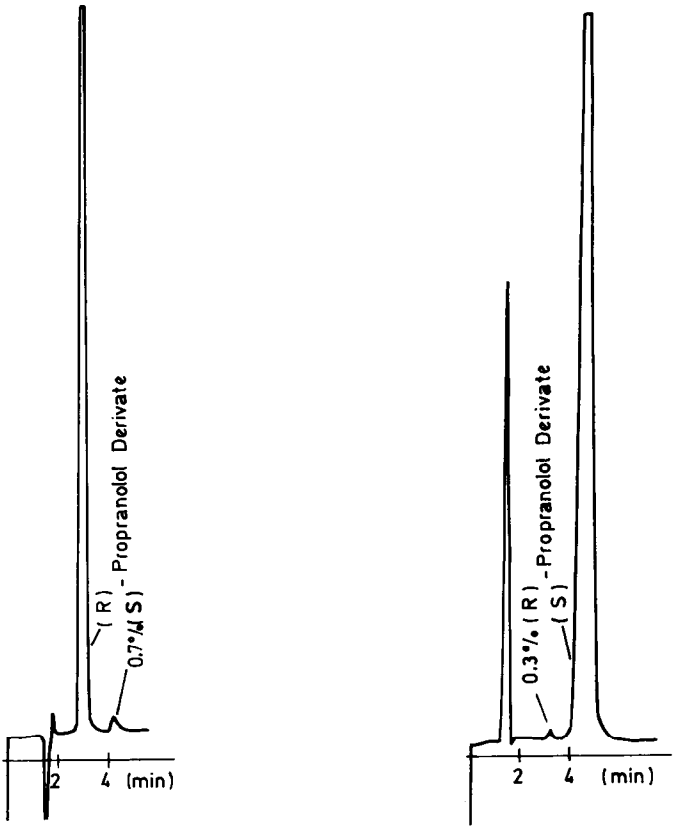


Fig. 3. HPLC separation of (*R,S*)-propranolol, derivatized with (*R,R*)-O,O-diacetyl tartaric acid anhydride. Conditions: column, 250 × 4.6 mm I.D., packed with Spherisorb ODS, 5 μm; mobile phase, 2% acetic acid in water (adjusted with ammonia to pH 3.7 in the final mixture)–methanol (50:50); flow-rate, 1.5 ml/min; detection, UV 254 nm.

substituents also fits into this argument. If the amine function significantly participates in the total molecular complex, its bulkiness should be important.

As is evident from Table VI, the *n*-propyl group shows, surprisingly, the highest increment in terms of "stereoselectivity", whereas the more bulky isopropyl and *tert.*-butyl groups have smaller ones. The free NH_2 group has the smallest effect on the overall "stereoselectivity", which is in accordance with the NMR studies showing hindered rotation of the N-alkyl substituents.

Finally, the value of the method described should be briefly discussed. The example of a typical chromatogram (Fig. 3) of a resolved pair of derivatized alkanolamine (*e.g.* propranolol) shows that peak ratios down to 1:1000, as well as the (*R*)-form in addition to the (*S*)-form or *vice versa*, can be resolved easily. From the fact that the enantiomeric purity of one antipode of the SA molecule could be detected down to 99.8%, it may be presumed that the optically active SE reagent has an optical purity higher than 99.9% and that virtually no racemization occurs during the derivatization and separation steps (*cf.* Introduction). The latter, very important criterion was verified by the following experiment with propranolol as an example. *R,S*-(±)-Propranolol was derivatized with DATAAN according to the procedure for larger amounts. The diastereomeric pairs of antipodes were separated on a preparative silica gel column with toluene–acetone (8:2) as eluent[25]. The first fraction corresponds to the (*R,R*)-O,O-diacetyl tartaric acid (*S*)-propranolol monoester, which was analysed by HPLC under the conditions given in Fig. 3. Thus, the actual *R*/*S* ratio of propranolol as its derivative is determined. By hydrolysis of this fraction to (*S*)-propranolol base, one should obtain an optical purity corresponding to the values obtained above, provided no racemization occurred during the hydrolysis. A repetition of the derivatization procedure with this purified (*S*)-(−)-propranolol and DATAAN (now on an analytical scale) and reevaluation of the ratio of the resulting diastereomeric pair showed that practically no racemization (less than 0.1%) is observable, at least under the conditions employed. This observation must be tested to determine whether it is also true for other (β-adrenergic active) alkanolamines than propranolol. However, because of their structural and chemical relationships, similar behaviour can be assumed.

To summarize, the described analytical and preparative indirect separation technique of enantiomeric aminoalcohols seems to be valuable in several respects and opens up a fairly sensitive method of checking the optical purity of alkanolamines of different structures.

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