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Tartaric acid derivatives as chiral sources for enantioseparation in liquid chromatography*

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Abstract: Based on a systematic review of the usage of tartaric acid as a chiral source to resolve enantiomeric compounds, a concept is presented for synthesizing new chiral stationary phases utilizing tartaric acid derivatives as the chiral selector. The results indicate that the conformational change of one of the three optically active centres of the chiral selector strongly affects its enantioselectivity for certain types of compound.

Keywords: *Chiral stationary phase based on tartaric acid; HPLC enantioseparation; tartaric acid derivatives.*

Introduction

Optically pure (R,R)- and (S,S)-tartaric acids are relatively cheap natural products and available in large quantities. Therefore it seems convenient to take these as starting materials to synthesize optically pure derivatives, and also to use tartaric acid (TA) as a source to stereoselectively resolve racemic compounds.

In brief, when using TA as a precursor for stereospecific synthesis two avenues are open in principle [1, 2]: (a) taking the chiral backbone of TA, either maintaining or converting the chiral centers, and directly synthesizing the final products with high optical purity; or (b) using TA derivatives as chiral catalysts which induce chirality with high enantiomeric excess in other compounds during specific synthetic routes.

However, the scope of this article is to discuss various stereoselective separation techniques by focussing exclusively on TA derivatives as a chiral source.

The methods employed to resolve racemates can be divided into two main types: (1) enantioseparations via non-covalently-bonded quasi-diastereomeric molecular complexes, termed the 'direct enantioseparation technique'; and (2) enantioseparations via the formation of covalently-bonded diastereomeric derivatives, termed the 'indirect enantioseparation technique'. Taking the latter technique first, the principal method is for a racemic compound (chiral selectand, SA) to be derivatized with an optically pure reagent (chiral selector, SE) to form a pair of antipodes, which as such are separable on diverse nonchiral chromatographic systems, but also by crystallization, for instance.

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Examples of this approach are described by Abe and Mushe [3], who used gas chromatography (GC) to separate (±)-menthone as its diastereomeric ketal with (R,R)-tartaric acid dimethyl-ester. Lindner *et al.* [4] recently described separations of diastereomeric tartaric acid mono-esters of racemic alkanolamines and betablockers, respectively; in this work astonishingly high stereoselectivity factors were observed for the separation of the diastereoisomeric pair.

For analytical purposes especially, the 'indirect enantioseparation technique' has several general limitations [5]. However, for separating and isolating enantiomeric compounds on the preparative scale, TA as a chiral source has great potential, considering also the often advantageous price-performance ratio. With respect to the 'direct enantioseparation techniques', it should be mentioned that with this general term numerous and mechanistically different enantioselective methods are covered [6, 7]. In this article only those techniques will be considered in which TA or derivatives thereof operate as chiral selectors (SEs).

It is generally considered that one needs at least three simultaneously-acting binding points between the chiral SE and the SA molecules; at least one of these binding points has to be stereospecific, to perform enantioseparation [8]. Along these lines, chromatographic enantioselective separation models in particular seem to fit into this model very well, as can be seen from the fundamental work of Davankov [6] and Pirkle [9]. However, looking at other enantioselective separation techniques such as, for instance, stereoselective ion-pair formation between chiral acids and bases, or stereoselective (helical) inclusion chromatography, it seems clear that the 'three-point binding rule' [8] should not be defined so strictly, because it does not consider the effects of chiral environments which can be created in various ways. Undoubtedly, it is not yet possible to discuss direct enantioseparation without involving any other chiral partner or source (molecule aggregations included) [10, 11].

In any attempt to systematize those direct enantioseparation techniques which involve TA derivatives, one arrives at the following groups of techniques.

Enantioseparation via salt formation between optically active partners

This process results in quasi-diastereomeric molecular complexes (ion pairs) which differ in their individual physicochemical properties, such as crystallization. This classical method is still widely used today, as can be seen from the literature referred to by Newman [12]. As a representative example, one can mention the discrimination of (R,S)-propranolol using *O,O*-ditoluoyl tartaric acid as resolving agent [13]. In principle this technique should also be transferable to chromatographic systems, as has been elegantly demonstrated by Pettersson and Schill [14]. Examples using TA derivatives as chiral counter ions have not yet been described.

Enantioseparation via stereospecific solvation

Solvation phenomena should also be considered as a form of molecular complex formation and therefore it can also be stereoselective by forming diastereomeric molecular aggregates, which differ in their overall physicochemical properties. Realizing this, Prelog *et al.* [15, 16] were able partially to resolve ephedrine-type compounds as their enantiomeric hexachlorophosphate ion pairs using (R,R)-tartaric acid di-*n*-butyl-ester as chiral source and chiral solvents, respectively.

Based on these observations, Pettersson and Stuurman [17] designed an enantioselective HPLC system by dynamically adsorbing the above-mentioned tartrate onto a

reversed-phase packing thereby succeeding in resolving some racemic ethanolamines. Recently these authors were also able to separate chiral tertiary amines (H. Stuurman, C. Petterson and E. Heldin, personal communication). Hence it follows that the diastereomeric binding model, as proposed by Prelog [15], has to be examined in some way, since the simple three-point binding rule via hydrogen-bonding does not fit these observations very well. More results and findings can be expected along these new and interesting lines.

Enantioseparation of chiral complexes and stereoselective ligand exchange mechanisms

Yoneda and coworkers [18] showed that positively-charged complexes of, for example, cobalt III with diene can be optically active and can be separated chromatographically with TA, which is coordinated to form diastereomeric molecule (chelate) complexes. In this example TA is not directly coordinated to the metal ion. However, TA derivatives are able to chelate transition metal ions directly, which leads to a second method, namely chiral ligand exchange chromatography, as already described by the authors (W. Lindner and I. Hirschböck, in preparation). With dynamically-coated chiral ligand exchange phases based on tartaric acid monoamides and using Cu^{II} as the complexing ion, the authors have been able to resolve all common α-amino acids as well as *N*-methylated amino acids. Using a covalently-bonded TA amide phase, racemic norepinephrine could also be resolved. A somewhat similar TA stationary phase was described recently, although no data on its chromatographic enantioselectivity were shown [19].

Direct chromatographic enantioseparation in non-aqueous systems

This approach takes advantage of dipole–dipole, π–π, hydrogen-bonding and hydrophobic interactions between chiral selectors, based on TA backbones and racemic SA molecules. Following the pioneering work of Pirkle *et al.* [9], it seems logical to the present authors to use as a chiral source TA which has been modified such that its final structure would fulfil the demands for the rather specific, but effective, binding mechanisms. As described below, this conclusion has been shown to be correct, in that the authors have designed new chiral phases such as CITAP-1 (reaction scheme, see Fig. 1), able to resolve for instance (D,L)-Leu-*O*-Me as its dinitrobenzene derivative.

Taking advantage of the reciprocal principle concerning SE and SA by reversing the π-acid and the π-base substituents in the SE and SA molecules, it should be possible to resolve on such chiral phases various types of compounds with different functionalities, but presumably substituted with aromatic rings. The extent to which these phases may differ as regards enantioselectivity from the so-called 'Pirkle phases', since the chiral SEs will have quite different conformations, has yet to be established and is the subject of continuing investigations.

Experimental

Apparatus

The LC pump used was a Model 410 (Kontron), the injector a Rheodyne Model 7120 with 20-μl loop, the detector a Model LC 15 (at 254 nm) (Perkin–Elmer). Thermostatic control was achieved by using a waterbath. The 'chiral columns' were of stainless steel, 200 × 4.6 mm i.d. and slurry-packed in the authors' laboratories. Optical rotation was measured with a Perkin–Elmer M 241 spectropolarimeter.

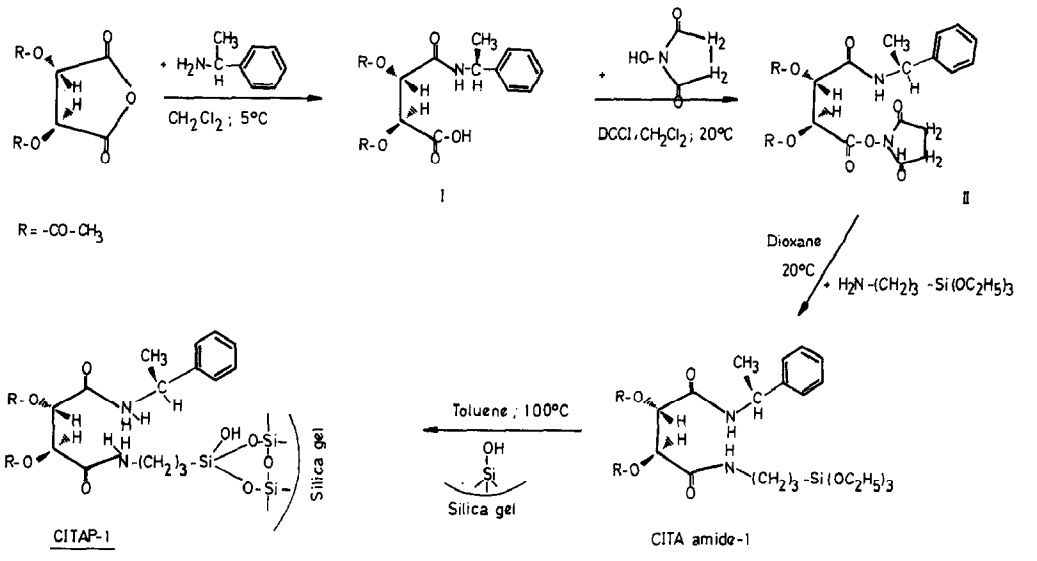

Figure 1
General reaction scheme for the synthesis of various chiral stationary phases based on tartaric acid derivatives.

Chemicals and reagents

The column packing material used was LiChrosorb Si 100 (7 μm) (Merck, Darmstadt, FRG). The solvents *n*-hexane, 2-propanol, cyclohexane, CH_2Cl_2, $CHCl_3$, toluene, methanol, acetic acid and aqueous ammonia, were all *pro analysis* grade and obtained from Merck. (R,R)-tartaric acid, acetyl chloride, (+)- and (−)-α-methylbenzylamine, *N*-hydroxysuccinimide and dicyclohexylcarbodiimide were obtained from Fluka (Buchs, Switzerland), and the 3-(trimethoxysilyl)-propylamine from Dynamit Nobel (FRG).

Preparation of (R,R)-O,O-diacetyl tartaric acid diamide bonded phase (CITAP-1)

Synthesis of the chiral N-(3-*triethoxysilylpropyl*)-N′-(*R*)-α-*methyl-(benzyl)-(R,R)*-O, O-*diacetyl-tartaric acid diamide (CITAamide-1)*. Under anhydrous conditions and at room temperature 25 g (0.11 mol) (R,R)-diacetyl-tartaric acid anhydride [20] was dissolved in about 300 ml CH_2Cl_2, to which 26.6 g (0.22 mol) (R)-α-methylbenzylamine, dissolved in 20 ml dry CH_2Cl_2, was dropped under stirring and ice-cooling for about 12 h. The CH_2Cl_2 phase was washed with dilute aqueous HCl containing NaCl. After extracting the organic phase with aqueous $NaHCO_3$, compound I dissolves into the aqueous phase; the organic phase was then discarded. After acidifying the aqueous phase with HCl to *ca* pH 2.0 and saturating it with NaCl, the resulting oil was re-extracted into CH_2Cl_2. After drying the organic phase with $MgSO_4$ it was evaporated to dryness, resulting in a crystalline product I; yield 21.5 g (57% of theory).

15.2 g (0.045 mol) of compound I was dissolved in 200 ml dry dioxane, 5.2 g (0.045 mol) *N*-hydroxysuccinimide was added and finally 9.3 g (0.045 mol) dicyclohexylcarbodiimide (dissolved in 20 ml dioxane). The reaction time was about 2 h at room temperature. After filtering off the dicyclohexyl urea the solution was evaporated to dryness. The oily residue was dissolved in $CHCl_3$, with water extracted, the organic phase dried with $MgSO_4$ and afterwards evaporated to dryness. The viscous yellowish oil crystallized by stirring with cyclohexane. The yield of product II was 14.4 g (73% of theory).

Product II was redissolved in 100 ml dry CH_2Cl_2 and treated for 3 h with an equimolar amount of 3-(triethoxysilyl)-propylsilane (7.3 g, 0.033 mol), by dropping it into the solution while stirring at room temperature. The mixture was evaporated to dryness and the remaining oily residue redissolved in toluene, whereby the recovered *N*-hydroxy-succinimide crystallized. After the crystals had been filtered off, the toluene solution was evaporated resulting in a yellowish oil, CITAamide-1, which was characterized by ^1H NMR; yield 11.0 g (67% of theory). No racemization was observed during the reaction.

Bonding reaction of CITAamide-1 onto silica gel. A 6 g amount of acid-washed silica gel (LiChrosorb Si 100, 7 μm), dried at 70°C, was suspended in 200 ml toluene to which 11 g (0.02 mol) chiral silane +1 g toluene–sulphonic acid were added and slowly stirred at 100°C for 12 h. The modified silica gel was washed with toluene, methanol, water and methanol (100 ml portions) by a sedimentation technique. The chiral phase (CITAP-1), yield 8 g, was vacuum-dried at 60°C for 24 h.

CITAP-2 was synthesized in the same way as CITAP-1, except that instead of (R)-α-methylbenzylamine, its antipode was incorporated into the chiral SE.

Characterization of the chiral phase. Each chiral phase was characterized by IR using a diffuse reflection technique (R. Dietel, A. Fuchsgruber and W. Lindner, in preparation) and found to be chemically identical with the IR spectra of CITAP-1 and CITAamide-1, respectively.

Results and Discussion

As can be clearly seen from the introduction, TA-derivatives show versatile possibilities to act as chiral sources to resolve optically active compounds. Beyond this, the present work introduces a new concept for chiral stationary phases, a route to synthesize them, and their application possibilities. The general reaction scheme for preparing such phases (see Fig. 1) has two main advantages, the first being the flexibility in terms of choosing different starting materials (TA derivatives) and amine components, combined with the fact that the final chiral silanes can be easily characterized.

The second advantage arises from the fact that the general problems arising when chemical reactions are performed on a solid surface (e.g. derivatizing an amine phase) can be avoided. Such reactions are hardly reproducible, always incomplete and have tendencies for racemization.

As a result of this concept new chiral stationary phases (CITAP-1) have been synthesized. The enantioselectivity of the CITAP-1 phase is demonstrated for DNB-D,L-Leu-*O*-Me in Fig. 2. Similar to Pirkle's binding models [9] π–π interactions combined with hydrogen bonding and/or dipole–dipole interactions can be considered as binding forces. The chiral information of the selector SE is not yet clear, since the selector contains three chiral centres. Their conformation and their spatial situation with respect to each other should strongly affect the accessibility of the binding points for the SA molecules.

Accordingly, it is interesting to note that even the conformational change of only one chiral group, the amide group, leads to a complete loss of enantioselectivity for a particular racemic compound, as can be seen from Table 1. The capacity factor (k') values are relatively similar, an indication of equal lipophilicity for both chiral phases. However, this is hard to separate from other binding forces responsible for retention,

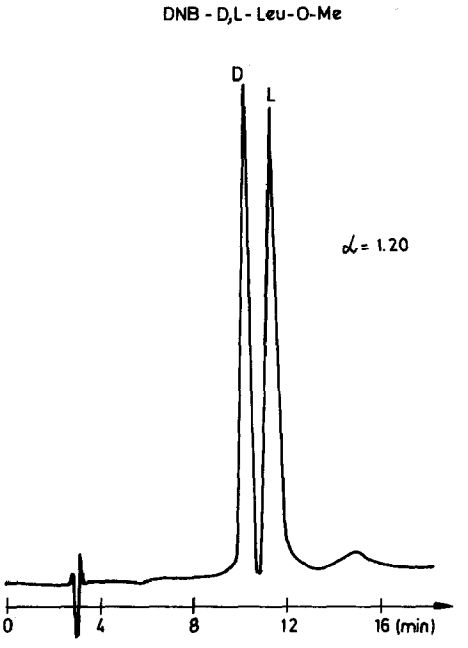

Figure 2
Enantioseparation of DNB-D,L-Leu-*O*-Me on CITAP-1. Conditions: Column 250 × 4.6 mm i.d. packed with CITAP-1 (7 μm); mobile phase, hexane–2-propanol (90:10, v/v); Flow rate, 2.0 ml/min; UV detection at 254 nm.

Table 1
Enantioselectivity of CITAP-1 and CITAP-2*

Solute	CITAP-1 k'_D	k'_L	$\alpha = \frac{k'_L}{k'_D}$	CITAP-2 k'_D	k'_L	$\alpha = \frac{k'_L}{k'_D}$
3,5 DNB-(D,L)-Leu-*O*-Me	2.4	2.9	1.20	2.4	2.4	1.0
3,5 DNB-(D,L)-Phegly-*O*-Me	7.5 sholder	8.0	1.07	7.7	7.7	1.0

* CITAP-2 differs from CITAP-1 only in the conformation of the α-methylbenzylamine group; the conformation of the TA backbone remains the same (see experimental). For chromatography i.e. conditions, see Fig. 2).

especially for such relatively rigid chiral phases as CITAP-1 and CITAP-2, respectively. For phases with chiral helical structures the retention properties are almost unpredictable.

The observations discussed above should demonstrate quite clearly that it is not sufficient only to make a chiral stationary phase to obtain resolution. It is moreover a necessity that one should be aware of all conformational phenomena involved, or at least one should try to obtain some deeper understanding of them. According to this, two more facts should be briefly mentioned. Firstly, the more optical active centres the SE and/or the SA molecules possess, the more complicated and unpredictable the binding mechanisms for enantioselectivity will be. Secondly, the more equally strong binding points there are available, forming different structured diastereomeric molecule complexes both in the SA and SE molecules, the more likely it is that efficient enantioseparation will fail. It is hoped that the present discussion may illustrate the

broad spectrum of possibilities available for the design of new chiral selectors. It should perhaps be noted that it is, however, all too easy to be disappointed.

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