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CINCHONA ALKALOIDS FOR PREPARING NEW, EASILY ACCESSIBLE CHIRAL STATIONARY PHASES

II*. RESOLUTION OF BINAPHTHOL DERIVATIVES ON SILICA-SUPPORTED QUININE

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SUMMARY

The preparation of the new chiral stationary phase SiSQuin, obtained by reaction of γ-mercaptopropylsilanized silica with the commercially available *Cinchona* alkaloid quinine, is described. This support is efficient for the rapid (6–7 min) separation of some binaphthol derivatives (separation factors between 1.08 and 1.16). The use of a circular dichroism detector allows the elution order to be established in a non-empirical way. This information is then used to propose a chiral recognition mechanism.

INTRODUCTION

The search for new chiral stationary phases for high-performance liquid chromatography (HPLC) is growing in importance[1–5]. In fact, problems such as the determination of enantiomeric composition and obtaining even small amounts of both of the antipodes of a racemate can, in principle, be solved for a variety of classes of chemical compounds if one or more versatile chiral stationary phases are available. It should be stressed that these problems are not confined to synthetic organic chemistry; the production and the stereochemical characterization of chiral compounds are becoming routine in inorganic chemistry, organometallic chemistry and catalysis (*e.g.*, ref. 6), and they are of great importance in pharmacology and biochemistry, as the biological properties of a compound are often allied to an enantiomeric form (*e.g.*, ref. 7). Since the first attempt[3] by Willstätter to resolve racemates by chromatography, considerable progress has been made and, at present, several chiral stationary phases are known[1–5] that are efficient in the resolution of several classes of racemates.

* For Part I, see ref. 8.

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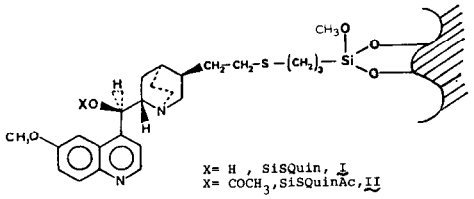


Scheme 1.

Recently, a new chiral stationary phase [SiSQuin, I (Scheme 1)] has been proposed[8] that can be easily obtained by derivatizing a silica support with the cheap, commercially available *Cinchona* alkaloid quinine. Quinine and some of the other *Cinchona* alkaloids have already been used to prepare[1] asymmetric sorbents, but the hydroxy group and the quinuclidine nitrogen were employed to form the bond with the insoluble support (a chloromethylated styrene–divinylbenzene copolymer). Here, for the first time, an alkaloid of this type has been covalently bonded to a silica support via the olefinic group. In this way, several functional groups (a secondary hydroxy group, two nitrogen atoms with different basic characteristics and a heteroaromatic ring) in a bulky chiral system could afford multiple[9] "contact" points with the racemate to be resolved, with the consequence of possible recognition between enantiomers.

In this paper, an analysis of the resolution of binaphthol derivatives on the new chiral stationary phase SiSQuin (I) is presented, including the direct determination of elution orders by means of a circular dichroism (CD) detector[10] and the proposal of a chiral recognition mechanism. Indeed, optically active binaphthols constitute an important class of compounds that have been used successfully as ligands in asymmetric synthesis[11], and consequently any method that can resolve them with known optical purity and determine their absolute configuration is of great interest.

EXPERIMENTAL

Chromatographic resolution

The separations were carried out using a Jasco Twincle apparatus. The absorption was obtained by means of a Uvidec-100V detector, whilst CD detection was effected with a Jasco J500C spectrometer equipped with a micro-HPLC cell.

Preparation of the chiral stationary phases SiSQuin (I) and SiSQuinAc (II)

Preparation of I. LiChrosorb Si 60 (Merck) (5 μm, 4.8 g) was treated with 20 ml of (3-mercaptopropyl)trimethoxysilane (Merck) in 20 ml of anhydrous pyridine–toluene (1:1). The slurry was heated at 90°C for 24 h. After centrifugation the solid was washed (toluene, acetone, diethyl ether, pentane) and dried under vacuum. This derivatized silica was suspended in chloroform and refluxed with quinine (Aldrich) (3.2 g) and α,α'-azobisisobutyronitrile (AIBN) (164 mg) as a radical source for 30 h. After centrifugation, the solid was washed exhaustively with methanol until the washings did not show any absorption of the quinine chromophore (325 nm). A 250 × 4.6 mm I.D. column was then slurry packed (carbon tetrachloride) with this material, using conventional techniques.

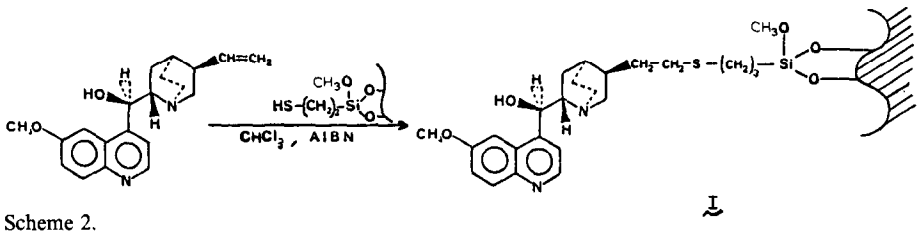
Preparation of II. To 10 g of 9-O-acetylquinine, prepared following known procedures[12], in 60 ml of anhydrous ethanol-free chloroform, were added under an inert atmospheric and with magnetic stirring 20 ml (21.5 g) of (3-mercaptopropyl)-trimethoxysilane pane-1-thiol and 0.449 g of AIBN. After reaction for 24 h with refluxing chloroform, the solvent and the thiol in excess were removed under vacuum. To the yellow solid obtained, dissolved in 20 ml anhydrous pyridine–toluene (1:1), were added 5 g of LiChrosorb Si 60 (5 μm). The slurry was maintained at 80°C for 24 h. After centrifugation and washing with methanol until the disappearance of the quinine chromophore (absorption at 325 nm) the solid was dried under vacuum. A 6.3-g amount of the chiral stationary phase II were obtained in this way.

Preparation of the binaphthol derivatives 2–6

These compounds were prepared following the procedures described by Pirkle and Schreiner.[13] The NMR spectra of the products were consistent with the expected structure.

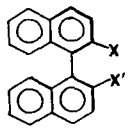
RESULTS AND DISCUSSION

The chiral stationary phase I can be conveniently prepared as follows. Treatment of LiChrosorb Si 60 (5 μm) with (3-mercaptopropyl)trimethoxysilane in dry benzene–pyridine affords γ-mercaptopropylsilanized silica, which can react with quinine in the presence of AIBN as a radical initiator, providing I. As expected, anti-Markovnikov addition of the thiol group to the double bond takes place with consequent formation of a thioether linkage, which allows the covalent bonding of the quinine residue to the insoluble support (Scheme 2).



Scheme 2.

Particular attention was made to evaluating the alkaloid content of I. This material was washed exhaustively with methanol until the quinine in excess (detected by its absorbance at 325 nm) was completely removed. Hydrolysis of a known amount of I with aqueous NaOH removes the chiral moiety from the support. Assuming that the ε_{325} for the hydrolysis product is the same as that for quinine, it was possible to establish that the quinine content of I was about 10% by weight, corresponding to 39 mmol of quinine per 100 g of silica. Preliminary attempts to resolve compounds 1–7 on I using hexane–2-propanol (90:10) as the mobile phase led to long analysis times (20–30 min) with no separation (as observed by UV detection at 230 nm) for all the above compounds, except 6 for which a value of the separation factor[5], α, of 1.03 has been measured. It is interesting, however, that a partial resolution of these compounds was actually obtained under these experimental conditions, as



1 : X = OH, X'=OH
2 : X = OH, X'=OCH_3
3 : X = OH, X'=O-*n*-C_4H_9
4 : X = OH, X'=O-dodecyl
5 : X = OH, X'=O-allyl
6 : X = OH, X'=O-iso-C_3H_7
7 : X = NH_2, X'=NH_2

Scheme 3.

shown by CD detection. This illustrates the usefulness of optical activity-based detectors in the chromatographic resolution of racemates using chiral stationary phases. In fact, the efficiency of a new support can be quickly judged even if UV detection gives a single peak. The appearance of two oppositely signed peaks in chiral detection suggests that the performance of the support is promising. Fig. 1 reports the resolution of compounds 5 and 6 as representative examples. In particular, the enantiomer having a positive CD at 235 nm (see below for a discussion about the choice of the wavelength for monitoring the resolution) is the first eluted antipode.

The use of the polar, aprotic eluent acetonitrile provided good separations of compounds 1–7 with very short analysis times. A typical resolution required 6–7 min. The results of these experiments are presented in Table I. Figs. 2 and 3 show examples of separations for compounds 3 and 5, respectively. It is clear that compounds 1–7 are separated very satisfactorily on chiral stationary phase I (SiSQuin), the values of the separation factor, α, being between 1.08 and 1.16. The monoalkylation of 1 reduces the capacity factor[5], k_1, which can be considered to be a measure of the achiral retention on the stationary phase, without reducing the α values. In contrast, the size of the alkyl group increases the separation (for 2, X' = OMe, α = 1.08; for 6, X' = O-isopropyl, α = 1.16). Information that could be very useful in understanding the recognition mechanism is a knowledge of the elution order of the two antipodes; this, in fact, is equivalent to knowing which enantiomer is more retained by the chiral stationary phase. Use of the CD detector provides this fundamental information, in principle. As has been shown[10] recently, it is possible to determine in a non-empirical way the elution order of the binaphthol enantiomers from a chiral column by recording the CD at a suitable wavelength and applying the exciton model. By using the low-energy coupling of the electrically allowed transitions of the β-naphthol chromophores of 1, it is possible to correlate a positive CD for this coupling mode (for which 235 nm is a representative wavelength and was then used to monitor the resolution process) and the *S* absolute configuration of 1. In this way it was possible to establish that the positive CD peak (at 235 nm) that is eluted first corresponds to the *S* enantiomer, *i.e.*, this antipode is less retained by the column. Moreover, all of the above considerations can be extended to the other derivatives, 2–7. The presence of different X' groups does not affect the spectroscopic characteristics (intensity, polarization direction) of the allowed transition of the ß-naphthol chromophore, and so the relationship between the CD and absolute configuration found for 1 is still valid. Hence it can be concluded that for all the other derivatives the first eluted (less retained) enantiomer has an *S* absolute configuration.

Another interesting aspect of this investigation is provided by the attempts to resolve the dialkyl derivatives of binaphthol. As is shown in Fig. 4, which reports the resolution of 2,2'-methoxy-1,1'-binaphthyl on chiral stationary phase I (SiSQuin), as a representative example, only a single peak is observable by UV detection, whilst

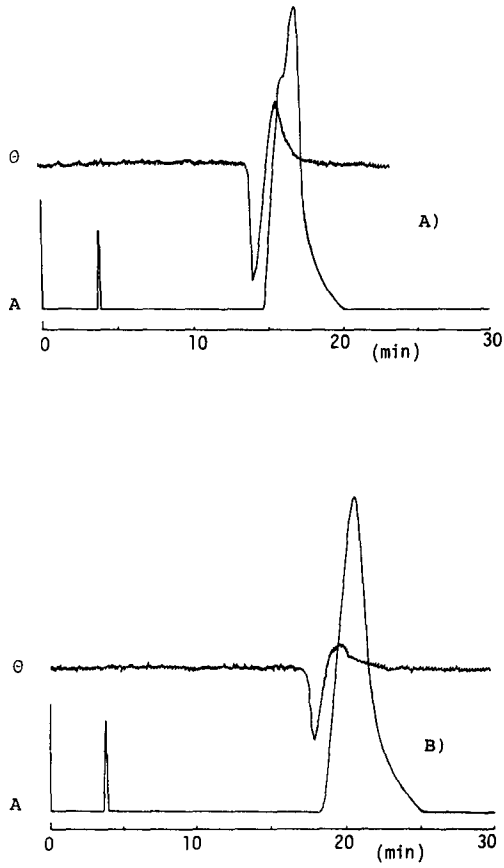


Fig. 1. Chromatographic resolution of (A) 5 and (B) 6 on I. Eluent, hexane–2-propanol (90:10); absorption, A (230 nm) and CD, θ (235 nm) detection. The first peak is chloroform, added as a retention marker.

TABLE I

RESOLUTION OF SOME BINAPHTHOL DERIVATIVES

Mobile phase: acetonitrile, flow-rate 1 ml/min.

Compound	k_1*	α	*CD sign***	*Absolute configuration****
1	3.9	1.10	+	*S*
2	1.0	1.08	+	*S*
3	0.9	1.11	+	*S*
4	1.3	1.10	+	*S*
5	0.8	1.09	+	*S*
6	0.7	1.16	+	*S*
7	1.0	1.12	+	*S*

* The values of the capacity factor are reported for the first-eluted peak.
** Sign of the CD at 235 nm for the first-eluted peak.
*** Absolute configuration of the first-eluted enantiomer.

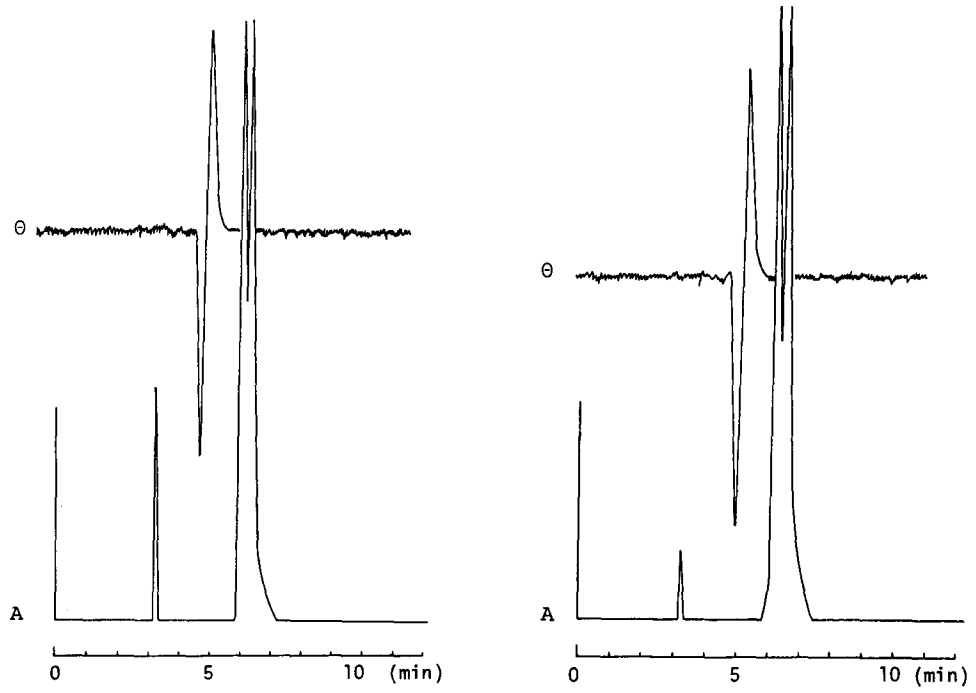
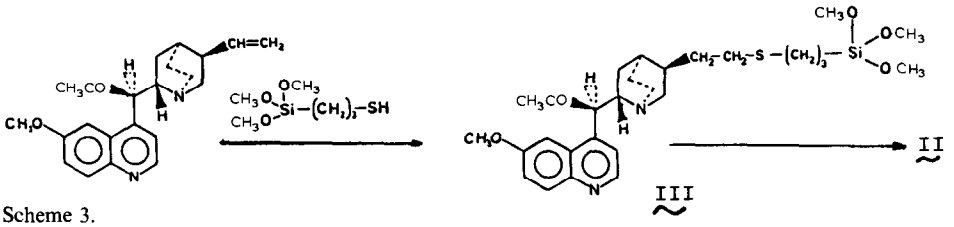


Fig. 2. Chromatographic resolution of 3 on I. Eluent, acetonitrile. Absorption, A (230 nm) and CD, θ (235 nm) detection. The first peak is chloroform, added as a retention marker.

Fig. 3. Chromatographic resolution of 5 on I. Conditions as in Fig. 2.

the CD detection at 235 nm shows two peaks of opposite sign, the positive one being eluted first. These facts indicate then that the chiral stationary phase gives rise to a small but definite separation of this compound and that, again, the first eluted enantiomer, showing a positive CD at 235 nm, has an *S* absolute configuration.

The separation of some of the compounds 1–7 has been also attempted on chiral stationary phase II (SiSQuinAc), where the hydroxy group on the C-9 of the quinine moiety is blocked by an acetyl function. This phase has been prepared following the procedure reported in Scheme 3; by reaction of O-acetylquinine, obtained by means of literature methods[12], with (3-mercaptopropyl)trimethoxysilane the adduct III can be obtained. III reacts with silica suspended in chloroform to afford the phase II. As reported in Fig. 5, which shows an attempt to resolve compound 3 on



Scheme 3.

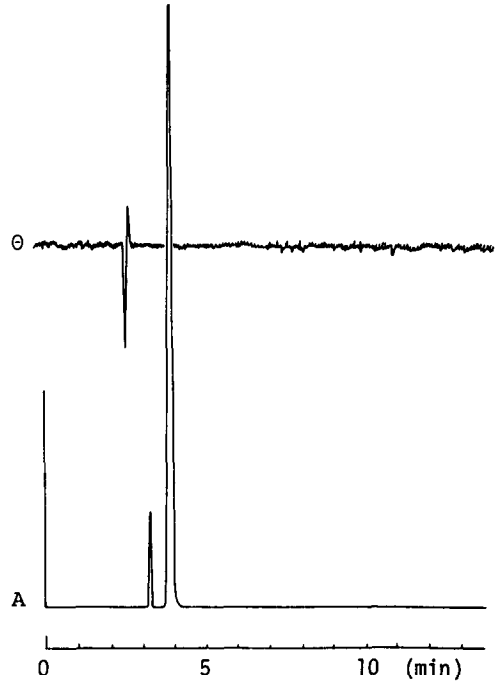


Fig. 4. Chromatographic resolution of 2,2′-methoxy-1,1′-binaphthyl on I. Conditions as in Fig. 2.

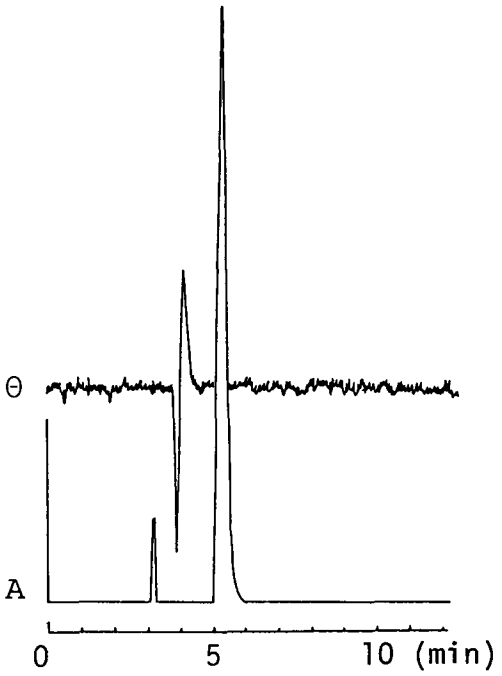


Fig. 5. Chromatographic resolution of 3 on II. Conditions as in Fig. 2.

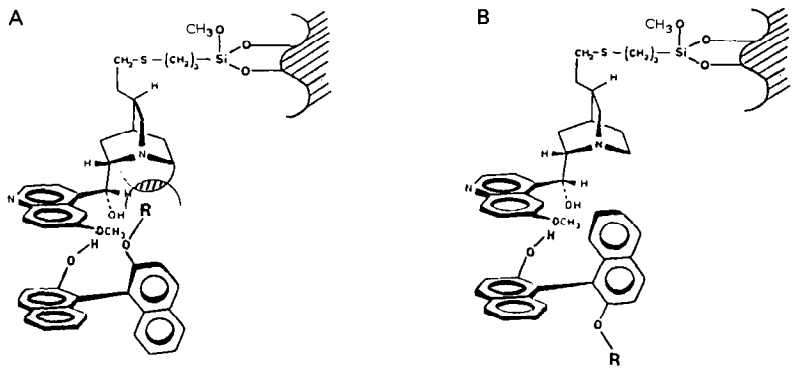


Fig. 6. Proposed chiral discrimination mechanism: possible interactions between I and the enantiomers of binaphthol derivatives. A, *S*-antipode; B, *R*-antipode.

this acetylated phase, UV detection affords a single, broad peak, whereas with CD detection two oppositely signed peaks can be recorded. Again, the first eluted peak shows a positive CD at 235 nm, so it corresponds to the *S* enantiomer. The low efficiency chiral stationary phase II in separating the above compounds in comparison with the non-acetylated phase I clearly reveals the importance of the free hydroxy group at C-9 in the latter in the chiral discrimination process. On the basis of all of the above experimental observations, a chiral discrimination mechanism can be tentatively proposed to account for the different interactions between the chiral stationary phase and the two antipodes of the substrate. Such a mechanism takes into account those interactions (such as hydrogen bonding, π–π forces and steric hindrance) that are normally considered[13] to be responsible for chiral recognition on optically active stationary phases. It is also consistent with a careful examination of molecular models. Fig. 6 shows the structure of chiral stationary phase I, where the quinine residue is placed in the same conformation that has been found[14] to be the most stable for the free quinine in solution, and the two possible interactions with the enantiomers of the binaphthol derivatives. As can be deduced from Fig. 6b, for the *R* enantiomer a hydrogen bond is possible between the hydroxy group at C-9 in the quinine nucleus of the chiral stationary phase and that on the binaphthol derivative. Also, there is a π–π interaction between the aromatic systems and at the same time the OR group can be placed in a spatial region that is relatively free. In contrast, for the *S* enantiomer, if the above hydrogen bonding and π–π interaction are to be mantained, the OR group must be located in a region that is fairly crowded, giving rise to considerable steric hindrance. Therefore, following this hypothesis the *S* enantiomer interacts in a weaker manner with the chiral stationary phase than does the *R* enantiomer, so the former is less retained and is eluted first, as is found experimentally.

CONCLUSIONS

This work has shown that quinine, the most common of the *Cinchona* alkaloids, can easily be used to derivatize γ-mercaptopropylsilanized silica to afford a chiral support that is efficient in the rapid resolution of racemic binaphthol deriva-

tives. Considering that the preparation of this chiral stationary phase is fairly simple and that quinine is an abundant, cheap, commercially available compound, it follows that the chiral support obtained is economic and readily accessible. Its efficiency in separating binaphthol derivatives is comparable to that of commercially available columns[13], which, however, generally require longer analysis times. In addition, the present phase appears to be stable, as after 4 months of continuous use the resolutions are completely reproducible.

Another interesting aspect of this work is the use of CD detection[10] at a suitable wavelength and the application of exciton theory to determine the elution order. The present procedure does not require partially resolved samples and/or compounds of known configuration to establish the elution order, but can be used directly on the racemic mixture. A knowledge of the configuration of the chiral support and the elution order allows us to propose a mechanism for the resolution. This is a general approach that can be applied to any situation to predict the recognition mechanism.

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