Journal of Chromatography, 398 (1987) 247–254
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 536

SEPARATION OF ENANTIOMERIC ACIDS USING IMMOBILIZED ACETYLQUININE AS A CHIRAL STATIONARY PHASE

CURT PETTERSSON* and CARLO GIOELI
Department of Analytical Pharmaceutical Chemistry, Biomedical Center, University of Uppsala, Box 574, S-751 23 Uppsala (Sweden)
(Received March 6th, 1987)

SUMMARY

Acetylquinine chemically bonded to silica was used as a chiral selector in reversed-phase chromatography for the separation of enantiomers of carboxylic acids and amino acid derivatives, *e.g.*, 2-(4-bromophenoxy)propionic acid, warfarin and N-benzoxycarbonylphenylalanine. An organic modifier (methanol) could be used to regulate the retention without significant influence on the stereoselectivity.

INTRODUCTION

The development of methods for the separation of enantiomers has opened up new possibilities to study the physiological activity of enantiomers. Optical antipodes can show quite different effects in biological systems, for instance when applied as drugs or herbicides[1,2]. The determination of the pharmacological or toxicological response of the individual enantiomers is of vital importance when evaluating new drug substances.

In liquid chromatography the enantiomers can be separated as diastereomeric derivatives, by use of chiral additives in the mobile phase or by use of chiral stationary phases[3,4]. A great variety of chiral stationary phases have been synthesized and evaluated for enantioselective separations. Chiral stationary phases which are based on the immobilization of small organic molecules, *e.g.*, (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine[5] or N-formyl-L-valylaminopropyl bound to silica[6], are generally used with organic mobile phases. Few stationary phases beside those based on proteins (albumin[7] or α-acid$_1$ glycoprotein[8,9]) give stereoselective interaction when used with aqueous mobile phases. The reversed-phase system has advantages in bioanalysis by allowing direct injection of aqueous samples, *e.g.*, plasma or urine.

Quinine and other cinchona alkaloids have been used for separation of enantiomers by fractional crystallization[10]. Quinine and its analogues have also been used as chiral counter ions for separation of enantiomers of carboxylic acids with organic mobile phases[11,12]. In 1954, Grubhofer and Schlieth synthesized a stationary phase with quinine as the chiral selector and applied it for separation of the enantiomers of mandelic acid with chloroform as the mobile phase[13]. Recently, Rosini *et*

0021-9673/87/$03.50

al.[14] described the use of a silica phase with immobilized quinine and organic mobile phases for the separation of enantiomers of binaphthol derivatives and arylalkylcarbinols[14].

In this study we have used acetylquinine bound to silica by a silanization reaction. The phase has mainly been applied in reversed-phase chromatography for the separation of enantiomeric carboxylic acids and amino acid derivatives. The influence of the mobile phase composition (pH, concentration and nature of organic modifier) on retention and stereoselectivity has been investigated.

EXPERIMENTAL

Apparatus

The pump was a Merck/Hitachi 655A-11 (E. Merck, Darmstadt, F.R.G.) and the detector a SpectroMonitor III (LDC, Riviera Beach, FL, U.S.A.) measuring at 254 nm. A Model 7120 syringe-loading injector (Rheodyne, Berkeley, CA, U.S.A.) was equipped with a 20-μl loop. The columns (100 mm × 3.0 mm I.D.) were of stainless steel with a polished inner surface, equipped with modified Swagelok connectors and stainless-steel frits (2 μm) (Altex, Berkeley, CA, U.S.A.).

The pH measurements were made with an Orion Research Model 801 and an Ingold combined electrode Type 401. The optical rotation was recorded with a Perkin-Elmer 214 polarimeter.

Chemicals

LiChrospher SI 100 (5 μm), methanol p.a., acetonitrile (LiChrosolv), *R*- and *S*-mandelic acid, *R*- and *S*-α-methoxy-α-trifluorophenylacetic acid were from Merck. Anhydrous quinine, dimethylchlorosilane, hexamethyldisilazane and hexachloroplatinic acid were obtained from Fluka (Buchs, Switzerland). *R*- and *S*-2-(4-bromophenoxy)propionic acid and racemic 2-(4-iodophenoxy)propionic acid were kindly supplied by the Department of Organic Pharmaceutical Chemistry, Uppsala University. *R,S*-2-(4-Chlorophenoxy)propionic acid, *R,S*-2-(3-chlorophenoxy)propionic acid, *R,S*-2-(2-chlorophenoxy)propionic acid and acetic anhydride were from Janssen Chimica (Beerse, Belgium). Haloxyfoc (2-4-[3-chloro-5-(trifluoromethyl)-2-pyridyloxy]phenoxy propanoic acid) was kindly donated by Professor G. Stenlid, Swedish University of Agricultural Sciences. N-Acetyl-D- and -L-tryptophan, N-benzoxycarbonyl-D,L- and -L-leucine, N-benzoxycarbonyl-D,L- and -L-valine, N-benzoxycarbonyl-L- and -D-phenylalanine, N-*tert.*-butoxycarbonyl-L- and -D-phenylalanine, dansyl-L- and -D-phenylalanine and *R,S*-warfarin were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.). The *R* and *S* forms of naproxen [2-(6-methoxy-2-naphthyl)propionic acid] were kindly supplied by Astra (Södertälje, Sweden). All other substances were of analytical or reagent grade and used without further purification.

Synthesis

Acetylquinine-I silica. The synthetic scheme is shown in Fig. 1. Quinine was converted into acetylquinine (A) using a standard procedure with acetic anhydride in pyridine. The purity of the product was estimated by thin-layer chromatography (TLC) to be better than 95% and $[\alpha]_D^{20} = -17.1$ ($c = 1.0$ in chloroform). The

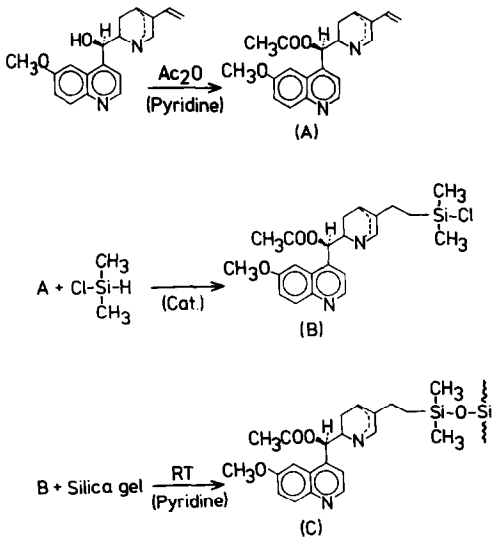

Fig. 1. Synthesis of the chiral stationary phase. Ac = Acetyl; RT = room temperature; Cat. = catalyzed.

conversion of the hydroxyl group was confirmed by IR and NMR spectroscopy.

A hydrosilylation reaction according to Speier *et al.*[15] was applied to synthesize the acetylquinine silane reagent (B). Dimethylchlorosilane (10 mmol) was added to a chloroform solution containing acetylquinine (A) (2.7 mmol) and hexachloroplatinic acid as a catalyst. The reaction mixture was refluxed for 2–6 h after which the excess of dimethylchlorosilane was evaporated *in vacuo*. For identification the acetylquinine silane reagent was hydrolysed in an aqueous methanol solution. The mass spectrometry (MS) and NMR spectra support the proposed structure of the silane reagent.

The acetylquinine silane reagent (B) (0.7 g) dissolved in pyridine was added to dry silica (4.5 g LiChrospher SI 100). The reaction mixture was gently rotated for 24 h at room temperature. The acetylquinine silica (C) was repeatedly washed with chloroform and methanol. Finally the product was dried at 60°C.

Acetylquinine-II silica. This phase was synthesized as above. After drying, the product was endcapped using hexamethyldisilazane. The endcapped silica was washed repeatedly with chloroform and methanol and finally dried at 60°C before being packed in the column.

Chromatographic technique

The columns were slurry-packed with an high-pressure pump using chloroform as suspending medium. They were washed with ethanol and deionized water before introducing the mobile phase. No recirculation of the mobile phase was used.

The buffer solutions had an ionic strength, I, of 0.1 M, unless stated otherwise. The mobile phases were prepared by mixing the buffer and organic solvent in specified volume ratios. All solutes injected were dissolved in the mobile phase. The volume of mobile phase in the column, V_m, was obtained from the front peak in the chromatogram.

All experiments were performed at room temperature (23°C).

RESULTS AND DISCUSSION

The basis for the different retentions of enantiomers on a chiral stationary phase is the formation of diastereomeric complexes with different stabilities. A three-point attachment due to attractive and/or repulsive interactions between the chiral stationary phase and at least one of the enantiomers is generally assumed to be required[5]. In this work, acetylquinine has been used as the chiral selector and the proposed structure of the stationary phase is given in Fig. 1 (C). Acetylquinine can give different interactions with solutes, such as hydrogen bonding, electrostatic interactions and charge-transfer interactions, which can be utilized to promote stereoselective retention.

Solute structure and stereoselectivity

The acetylquinine silica phase was applied for the separation of enantiomers of halogen-substituted phenoxypropionic acids, Table I. The nature and the position of the halogen substituent in the benzene ring influences the stereoselective interaction with the chiral selector. 2-(4-Iodophenoxy)propionic acid showed a significantly higher stereoselectivity than the corresponding chloro derivative, which might be due to steric effects. Similar observations have been made in systems with quinine as the chiral counter ion in an organic mobile phase, although the stereoselectivity in those systems was higher[12]. The decrease in stereoselectivity when the chloro atom is in *ortho* position may also be due to steric effects, the substituent preventing a close contact with the acetylquinine molecule. A complete resolution of *R*- and *S*-2-(4-bromophenoxy)propionic acid is shown in Fig. 2. The chromatographic performance was relatively good, with a reduced plate heighth, $h = H/d_p$, of 20 and an asymmetry factor of about 1.7. A new herbicide, haloxyfoc, could also be resolved into two enantiomeric forms, Table I.

The acetylquinine phase can also be used for separation of some enantiomers of amino acid derivatives, Table II. The data indicate that the bulkiness of the substituents attached to the nitrogen affects the stereoselectivity. *tert.*-Butoxycarbonyl (Boc)-phenylalanine shows a lower stereoselectivity than the corresponding benzoxycarbonyl (CBZ) and dansyl derivatives. CBZ-Valine in which an isopropyl group is

TABLE I

SEPARATION OF ENANTIOMERS OF HALOGENATED PHENOXYPROPIONIC ACIDS

Solid phase: acetylquinine-II silica. Mobile phase: acetate buffer (pH 5.34, I = 0.1 M)–methanol (6:4). k'_1 = Capacity factor for the first enantiomer eluted; α = ratio of k' for the second enantiomer eluted to k' for the first enantiomer eluted.

Acid	k'_1	α
2-(2-Chlorophenoxy)propionic	5.21	1.09
2-(3-Chlorophenoxy)propionic	5.75	1.12
2-(4-Chlorophenoxy)propionic	5.36	1.15
2-(4-Bromophenoxy)propionic	6.39	1.17
2-(4-Iodophenoxy)propionic	8.54	1.21
Haloxyfoc	35.7	1.12

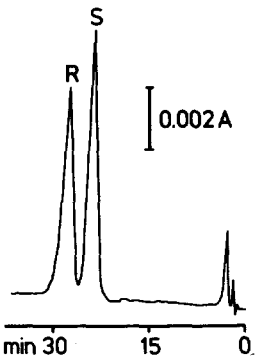

Fig. 2. Resolution of *R*- and *S*-2-(4-bromophenoxy)propionic acid on acetylquinine-II silica. Mobile phase: acetate buffer (pH 5.12, I = 0.05 *M*)–methanol (7:3).

attached to the asymmetric centre gave somewhat higher stereoselectivity than CBZ-leucine. The increase of one methylene group had a slight effect on the retention ($\Delta \log k' = 0.44$), *cf.*, valine and leucine in Table II.

Other acids with enantioselective retention on acetylquinine silica are given in Table III. Most of the acids that showed different retention times for the enantiomers contained besides the carboxylic group a second polar function in the vicinity of the chiral centre. However, naproxen (3) has a polar group situated far from the asymmetric carbon atom and warfarin (4) has an acidic enol and other polar groups not directly attached to the asymmetric carbon atom (Fig. 3). A partial separation of the two enantiomers of warfarin using the acetylquinine column is shown in Fig. 4.

Regulation of retention and stereoselectivity

As expected the "endcapped" acetylquinine silica phase gave higher retention than the "non-capped" phase. The retention could be decreased by increasing the amount of methanol in the mobile phase as shown in Table IV. The change in methanol content only slightly affects the stereoselectivity. A minor increase in stereoselectivity was noted for mandelic acid and naproxen as the methanol content increased from 30 to 50%.

Replacement of methanol by acetonitrile in the mobile phase gave rise to a slight decrease in stereoselectivity, Table V, and as expected a lower retention.

TABLE II

SEPARATION OF ENANTIOMERS OF AMINO ACID DERIVATIVES

Solid phase: acetylquinine-I silica. Mobile phase: acetate buffer (pH 4.92, I = 0.02 *M*)–methanol (85:15). Dansyl = 5-Dimethylaminonaphthalene-1-sulphonyl.

Solute	k'_1	α
CBZ-Leucine	15.3	1.04
CBZ-Valine	12.5	1.09
CBZ-Phenylalanine	26.3	1.10
Boc-Phenylalanine	12.4	1.06
Dansylphenylalanine	67.2	1.10

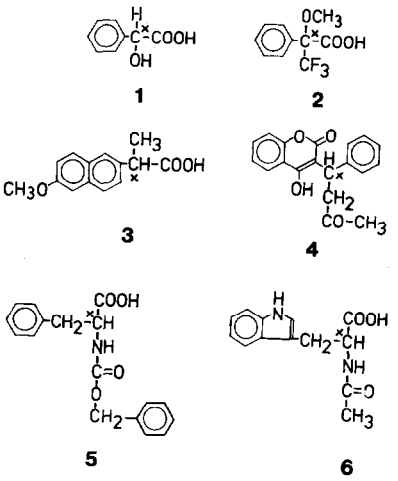

Fig. 3. Structures of solutes (see Table III). (x = asymmetric centre).

A further possibility to regulate the retention is to change the degree of ionization of the solutes by changing the pH in the mobile phase. A decrease in pH from 6.5 to 2.2 gave strongly increased retention for 2-(4-bromophenoxy)propionic acid and α increased from 1.06 to 1.10. The enantiomers of mandelic acid were not separated in systems with phosphate buffer, as was found with acetate buffer.

Preliminary experiments have indicated that the nature of the buffer ions as well as the ionic strength affect the stereoselectivity. The acidity constants for quinine in aqueous solutions are 4.1 and 8.0[16] and it is probably that a change in mobile phase pH will also affect the charge of the chiral stationary phase. However, further studies are necessary in order to elucidate the reason for the change in retention and selectivity when changing pH.

A decrease in retention time of about 10% during 1 week is generally observed with the acetylquinine phases.

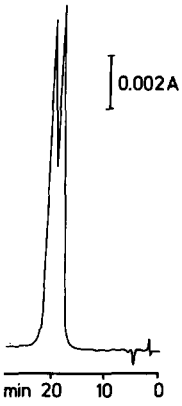

Fig. 4. Separation of *R*- and *S*-warfarin on acetylquinine-II silica. Mobile phase: acetate buffer (pH 5.34, I = 0.1 *M*)–methanol (6:4) (order between *R* and *S* enantiomers not determined).

TABLE III

SEPARATION OF ENANTIOMERIC ACIDS

Solid phase: acetylquinine-II silica. Mobile phase: acetate buffer (pH 5.34, I = 0.1 M)–methanol (6:4).

*No.**	*Solute*	k'_1	α
1	Mandelic acid	1.26	1.02
2	α-Methoxy-α-trifluoromethyl-phenylacetic acid	5.21	1.03
3	Naproxen	14.5	1.04
4	Warfarin	15.0	1.09
5	CBZ-Phenylalanine	16.6	1.08
6	N-Acetyltryptophan	2.61	1.09

* Structures as in Fig. 3.

TABLE IV

INFLUENCE OF METHANOL CONTENT ON RETENTION AND STEREOSELECTIVITY

Solid phase: acetylquinine-II silica. Mobile phase: acetate buffer (pH 5.2, I = 0.1 M)-methanol.

Solute	*% Methanol*					
	30		*40*		*50*	
	k'_1	α	k'_1	α	k'_1	α
2-(4-Bromophenoxy)-propionic acid	10.2	1.17	7.50	1.17	3.86	1.17
Mandelic acid	1.30	1.02	1.26	1.02	0.96	1.04
Naproxen	35.5	1.01	14.5	1.03	5.43	1.03
CBZ-Phenylalanine	42.0	1.06	16.6	1.08	6.14	1.07

TABLE V

INFLUENCE OF THE MODIFIER ON RETENTION AND STEREOSELECTIVITY

Solid phase: acetylquinine-I silica. Mobile phase: acetate buffer (pH 5.0, I = 0.02 M)–modifier (85:15).

Acid	*Modifier*			
	Methanol		*Acetonitrile*	
	k'_1	α	k'_1	α
2-(4-Chlorophenoxy)propionic	3.0	1.11	2.2	1.07
2-(4-Bromophenoxy)propionic	3.7	1.12	2.6	1.10
2-(4-Iodophenoxy)propionic	5.8	1.15	3.4	1.11

ACKNOWLEDGEMENTS

We are grateful to Professor D. Westerlund for valuable comments on the manuscript. The authors wish to thank Dr. U. Hackzell for help with the interpretation of the MS and NMR spectra. This work was supported by the Swedish National Board for Technical Development (project No: K-Ku 1972-102). The award of research grants to one of us (C.P.) from the Swedish Academy of Pharmaceutical Sciences and the I.F. Foundation for Pharmaceutical Research is gratefully acknowledged.

REFERENCES

1 M. Simonyi, *Medicinal Res. Rev.*, 4 (1984) 359.
2 E. J. Ariens, W. Soudijn and P. B. M. W. M. Timmermans (Editors), *Stereochemistry and Biological Activity of Drugs*, Blackwell, Oxford, 1983.
3 W. F. Lindner and C. Pettersson, in I. W. Wainer (Editor), *Liquid Chromatography in Pharmaceutical Development, An Introduction*, Aster, Springfield, OR, 1985.
4 S. Hara and J. Cazes (Editors), Optical Resolution by Liquid Chromatography, *J. Liq. Chromatogr.*, 9 (1986).
5 W. H. Pirkle and J. Finn, in J. D. Morrison (Editor), *Asymmetrical Synthesis*, Vol. 1, Academic Press, New York, 1983.
6 A. Dobashi, Y. Dobashi and S. Hara, *J. Liq. Chromatogr.*, 9 (1986) 243.
7 S. Allenmark, *J. Liq. Chromatogr.*, 9 (1986) 425.
8 J. Hermansson and M. Eriksson, *J. Liq. Chromatogr.*, 9 (1986) 621.
9 G. Schill, I. W. Wainer and S. A. Barkan, *J. Liq. Chromatogr.*, 9 (1986) 641.
10 S. H. Wilen, in *Topics in Stereochemistry*, Vol. 6, Wiley-Interscience, New York, 1971, p. 107.
11 C. Pettersson and K. No, *J. Chromatogr.*, 282 (1983) 671.
12 C. Pettersson, *J. Chromatogr.*, 316 (1984) 553.
13 N. Grubhofer and L. Schieth, *Hoppe-Seyler's Z. Physiol. Chem.*, 296 (1954) 262.
14 C. Rosini, C. Bertucci, D. Pini, P. Altemora and P. Salvadori, *Tetrahedron Lett.*, 26 (1985) 3361.
15 J. L. Speier, J. A. Webster and G. H. Barnes, *J. Am. Chem. Soc.*, 79 (1957) 974.
16 J. A. Dean (Editor), *Lange's Handbook of Chemistry*, McGraw-Hill, New York, 1979, pp. 5–23.