Journal of Chromatography, 365 (1986) 73–88
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 897

CHIRAL SEPARATIONS OF CATIONIC AND ANIONIC DRUGS ON AN α_1-ACID GLYCOPROTEIN-BONDED STATIONARY PHASE (ENANTIOPAC®)

II. INFLUENCE OF MOBILE PHASE ADDITIVES AND pH ON CHIRAL RESOLUTION AND RETENTION

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SUMMARY

The influence of mobile phase additives and pH on chiral resolution and retention on a high performance liquid chromatographic chiral stationary phase composed of α_1-acid glycoprotein bonded to diethylaminoethyl silica (EnantioPac®) has been investigated. Cationic and anionic compounds of widely differing structures were chromatographed and drastic effects on stereoselectivity were observed with hydrophobic charged modifiers. For cationic solutes, a decrease in the pH of the mobile phase from 7.0 to 6.0 gave reduced retention and, in some cases, improved selectivity when tetrabutylammonium or tetrapropylammonium bromide was used as modifier. For anionic solutes, a pH decrease from 6.6 to 6.1 gave enhanced retention but without a significant change in stereoselectivity. The steric bulk and hydrophobic moieties of the solute seem to have a strong influence on chiral selectivity. Widely different separating efficiencies were obtained with molecules of different structures.

INTRODUCTION

Analytical methods for separation and determination of enantiomeric compounds are often of vital importance in the development of new drugs and new therapeutic principles. During latter years, such studies have been significantly promoted by the development of a series of new separation methods based on the formation of diastereomeric complexes in the mobile or stationary phase of a chromatographic system.

Chromatographic systems with the enantiomeric complexing agent (the chiral selector) bound to a solid phase that can be used with aqueous mobile phases are of particular interest in the pharmacological studies of enantiomeric compounds. The

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mobile phases are compatible with biological fluids, and work-up procedures such as extraction and derivatization of ionized molecules are unnecessary.

One of the most promising of these phases has been developed by Hermansson[1,2] and is now commercially available as EnantioPac® (LKB, Bromma, Sweden). The chiral selector is α_1-acid glycoprotein (AGP) ionically bound to diethylaminoethyl silica and cross-linked by a procedure that involves oxidation, Schiff base formation and reduction of the enamines to secondary amines[3]. AGP is the main cationic binding protein in the human organism and it has an isoelectric point of 2.7 in phosphate buffer. It is composed of a peptide chain containing 181 amino acid units and five carbohydrate units which include fourteen residues of sialic acid. The carbohydrate units comprise 45% of the molecular weight, which is 41 000[4].

The stereoselective binding of cationic molecules to AGP in aqueous media has been studied by a dialysis technique using propranolol as the substrate[5,6]. Hermansson[7] has also determined the binding constants of the enantiomers of some cationic drugs by a chromatographic technique with a non-chiral solid phase and AGP present in the mobile phase. However, very little is known about how AGP binds ionized molecules. It seems that sialic acid is involved for some compounds, since Pike *et al.*[8] found that enzymatic desialylation of AGP reduces the binding affinity for cationic molecules while the binding of neutral and anionic molecules is unaffected.

EnantioPac appears to have a wide applicability to the resolution of molecules of pharmacological interest[2,9,10]. In our last paper[10], we demonstrated that the AGP-bonded column can be applied to chiral separations of cationic compounds of widely different structures, from simple aminoalcohols like ephedrine and pseudoephedrine to polycylic compounds such as methorphan and atropine. The retention and chiral selectivity is highly dependent on temperature and pH. Charged and uncharged modifiers will affect the retention, and in many cases significant improvements of the chiral separations could be obtained by the addition of hydrophobic, ionized mobile phase additives such as tetrabutylammonium bromide and octanoic acid. The conditions for the separation and chiral resolution of over 40 cationic molecules of pharmacological interest were presented.

This paper presents studies of the influence of mobile phase additives and pH on the chiral resolution and retention of cationic and anionic compounds of widely different structures. The often dramatic effect of the hydrophobic charged modifiers is demonstrated by studies on diastereomeric compounds and groups of closely related substances. The influence on the chiral selectivity of the steric bulk and hydrophobic moities of the solute is also illustrated, as is the widely different separating efficiency obtained with molecules of different structures. On the basis of these observations, the background to the large variations of the binding properties of the chiral phase with changes in the mobile phase composition and solute structure is discussed.

EXPERIMENTAL

Apparatus

The chromatographic experiments were performed with Spectra-Physics (Santa Clara, CA, U.S.A.) Model 8700 liquid chromatographs equipped with Spectra-

Physics Model 770 and Model 8773XR variable-wavelength detectors. The temperature of the columns and the mobile phase reservoirs was regulated by using thermostatically controlled circulating water. The injectors were Rheodyne Model 7125 with 10-μl loops.

The separations were performed on commercially available EnantioPac columns (100 mm × 4.0 mm I.D.) containing cross-linked α_1-acid glycoprotein bound to diethylaminoethyl silica (180 mg/g). The columns were generously supplied by LKB.

Materials

The terbutaline and bambuterol analogues were supplied by Draco (Lund, Sweden), UH 106 and 104 by Astra Pharmaceuticals (Sodertalje, Sweden) and nadolol A and B by Squibb (Princeton, NJ, U.S.A.). All other solutes were obtained from the stores of the U.S. Food and Drug Administration (Washington, DC, U.S.A.). The structures of some compounds of particular interest are given in Fig. 1.

The tetrapropylammonium and tetrabutylammonium bromides, aspartic, butyric and octanoic acids, ethylene and propylene glycols, 1,2-butanediol, N,N-dimethylethylamine, L-2,4-diaminobutyric and octylsulfate were purchased from Aldrich (Milwaukee, WI, U.S.A.). Decanoic and 6-aminohexanoic acids were purchased from Fluka (Hauppage, NY, U.S.A.). The 2-propanol was high-performance liquid chromatographic (HPLC) grade from Burdic & Jackson (Muskegon, MI, U.S.A.). All other chemicals were reagent grade and used without further purifications.

Chromatographic conditions

The standard conditions used during the chromatography were a flow-rate of 0.30 ml/min and a mobile phase temperature of 20.0°C. The mobile phases were 0.02 *M* phosphate buffers to which modifiers were added. The pH was adjusted to the desired level by the addition of sodium hydroxide or phosphoric acid. In most cases, a UV absorption maximum of the solute was chosen as the wavelength of detection. During most of the chromatographic studies, an EnantioPac column was placed in series before the injector to prevent changes in the properties of the separation column.

RESULTS AND DISCUSSION

Influence of solute structure

In our previous paper on chiral separations of organic cations on the EnantioPac column[10], we used a working hypothesis implying that the stereoselectivity (α) is dependent upon the presence of hydrogen bonding groups (HB), large substituents or cyclic structures at the ammonium ion, N^+, and/or the HB group and the distance between the HB and N^+. This was illustrated using two series of compounds, one related to metoprolol and the other related to tocainide. Separation factors and separation conditions were also given for about 40 other compounds, all containing more or less strongly hydrogen bonding groups such as alcohol, amide, ester, ether, indene, oxo, phenol, pyridine and phenothiazine moieties.

The applicability of the chiral phase is, however, so large that these require-

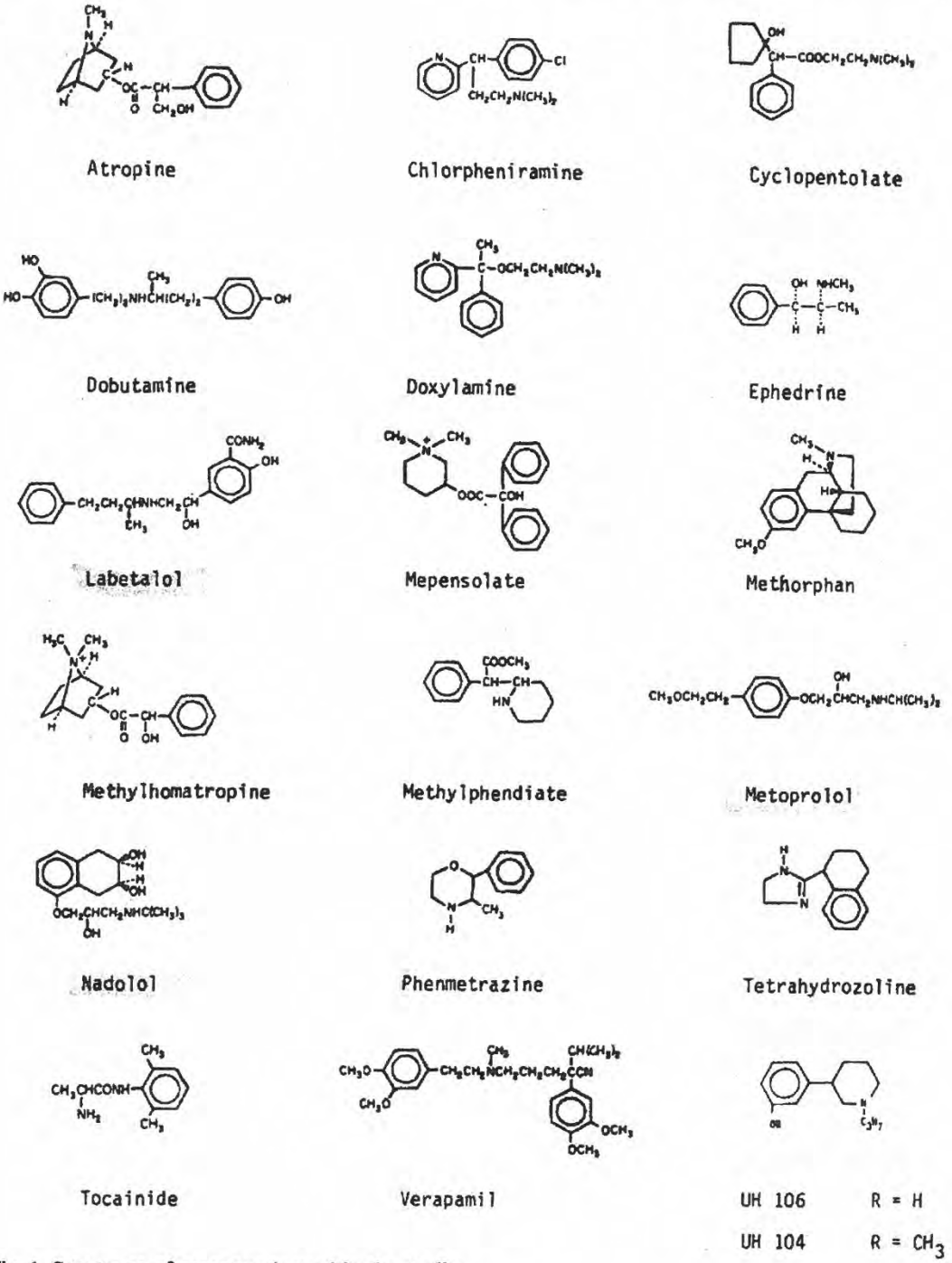

Fig. 1. Structures of compounds used in the studies.

ments can be considered only as basic observations on important structural features. More specific rules for the relationship between structure and chiral selectivity can be given only for groups of closely related compounds.

Further studies on cations containing several rings or ring systems have indi-

TABLE I

SEPARATION OF ENANTIOMERIC CATIONS

Mobile phase: modifier in 0.02 *M* phosphate buffer. k'_1 = capacity ratio of first eluted enantiomer; α = separation factor (k'_2/k'_1).

Solute	*Modifier*	*pH*	k'_1	α
Dobutamine	Tetrapropylammonium bromide (0.001 *M*)	6.0	14	1.56
Methorphan	Tetrabutylammonium bromide (0.003 *M*)	6.0	5.2	2.69
Tetrahydrozoline	Dimethylethylammonium (0.1 *M*)	7.0	5.8	1.66
Verapamil	Decanoic acid (0.005 *M*) + Tetraethylammonium bromide (0.05 *M*)	7.0	13	1.75
UH 104	Decanoic acid (0.01 *M*)	7.0	6.4	1.42
UH 106	Decanoic acid (0.01 *M*)	7.0	9.3	1.30

cated that the presence and position of the hydrogen bonding groups is of minor importance for the chiral selectivity of such molecules. Some examples are given in Table I. The bulky and very weakly hydrogen bonding compounds, tetrahydrozoline and methorphan, show high separation factors in systems with a cationic modifier in the mobile phase. The closely related compounds UH 106 and 104 give a further illustration: a change from a methoxy substituent in UH 104 to a phenolic group in UH 106 gives rise to a decrease in α.

In the previous paper[10], we also demonstrated that under certain conditions both anionic and cationic enantiomeric compounds can be resolved on the Enantio-Pac column. Further examples of the chiral resolution of anionic compounds are given in Table II. Resolution can be obtained with quaternary ammonium modifiers as well as with uncharged modifiers, but the former give higher separation factors.

It is important to notice that 2-phenylbutyric, 3-phenylbutyric and 2-phenylpropionic acids contain no hydrogen bonding moieties and that the separation factor increases with increasing length of the alkyl chain coupled to the chiral carbon. The results in Table II also indicate that the separation factor increases in the presence of a hydrogen bonding group and with increasing bulk of the aromatic group (*cf.* 2-phenylpropionic acid, 2-phenoxypropionic acid and ibuprofen).

It must be emphasized that the relationship between solute structure and chiral selectivity can be drastically changed by the properties of a modifier added to the mobile phase. Some examples of this are given in Table III, which contains the results for aminoalcohols related to terbutaline and bambuterol when decanoic acid and 6-aminohexanoic acid were used as modifiers. The chiral selectivity changes dramatically with the substitution in the aromatic ring, but in quite different directions with the two modifiers. For the diphenols, the highest separation factors are achieved with 6-aminohexanoic acid as modifier, while the monophenols have significantly higher separation factors in the presence of decanoic acid. It should be noted that no chiral resolution is obtained when there is a methyl substituent at the chiral carbon.

The bulkiness of the molecule seems to be of vital importance for the chiral resolution. Terbutaline with a tertiary butyl substituent at N^+ is easily resolved. However, it has not, as of yet, been possible to obtain a chiral resolution for metaproterenol (with an isopropyl substituent at N^+) or any other phenylethanolamine

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