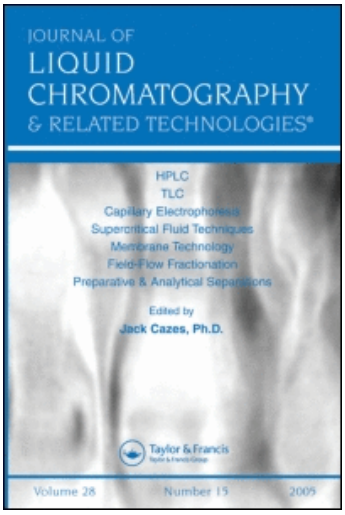

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SIMPLE CHIRAL LIQUID CHROMATOGRAPHIC ENANTIOSEPARATION OF SOME RACEMIC ANTIEPILEPTIC DRUGS

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ABSTRACT

Cellulose tris (4-methylbenzoate) known as Chiralcel OJ chiral stationary phase (CSP) has been successfully used for the enantioseparation of three clinically used antiepileptic drugs namely, mephobarbital, mephenytoin and benzonal. The chemical structure-chiral recognition mechanisms related to the enantiomeric elution order of these drugs are discussed.

INTRODUCTION

Mephobarbital (I), mephenytoin (II) and benzonal (III), the chemical structures of which are shown in Figure 1, are antiepileptic drugs that are clinically administered as racemates. Several studies showed that both mephobarbital (1-4) and mephenytoin (5) are metabolized stereoselectively in the body.

The resolutions of the enantiomers of I-III were achieved by different chromatographic methods using chiral gas chromatography (GC), (6,7), chiral high performance liquid

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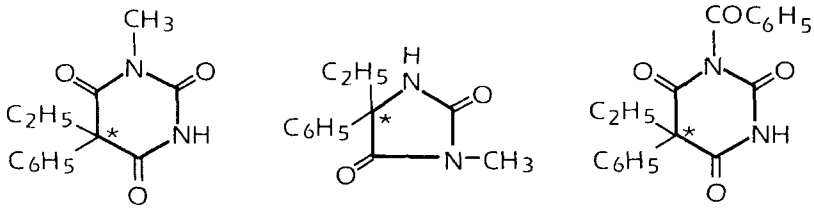

I Mephobarbital II Mephenytoin III Benzonal

FIGURE 1. The structure of the antiepileptic drugs studied. Asterisk indicates the position of the chiral carbon.

chromatography (HPLC), on a variety of chiral stationary phases (8-18). The resolution of the enantiomers of these drugs was also effected by using chiral selector β-cyclodextrin and its derivatives by thin layer chromatography (TLC) (19), HPLC (20-24) and displacement chromatography (25) techniques. Cellulose-based chiral stationary phases (CSP's) are often used in resolution of drug enantiomers (26, 27).

Described here is a simple, isocratic method for the enantioseparation of the racemates of these antiepileptic drugs I-III. Effective resolution was achieved on cellulose tris (4-methyl benzoate) chiral stationary phase, namely known as Chiralcel OJ column. Furthermore, preliminary evaluation of the chemical features involved in the enantioseparation of these structurally related drugs is discussed.

EXPERIMENTAL

Apparatus:

The HPLC system consisted of a Bio-Rad 1350 solvent delivery pump, a Rheodyne model 7125 injector and a Lambda Max Model 481 LC spectrophotometer UV detector, operated at 254 nm. The stationary phase of Chiralcel OJ column (25 cm x 0.46 cm I.D., coated on silica gel of particle size 10 μm) was purchased from Daicel Chemical Industries, Tokyo, Japan.

Chemicals:

Racemic mephobarbital (5-ethyl-1-methyl-5-phenylbarbituric acid), racemic mephenytoin (5-ethyl-3-methyl-5-phenylhydantoin) and racemic benzonal (1-benzoyl-5-ethyl-5-

phenylbarbituric acid) were generously supplied by Professor J. Bojarski, Nicolaus Copernicus Academy of Medicine, Krakow, Poland. Samples for (-)-R-mephobarbital and (-)-R-mephenytoin were kindly obtained from Professor J. Knabe, University of Saarland, Saarbrucken, Germany.

Determination of Enantiomeric Elution Order

The enantiomeric elution order was determined by chromatographing the (-)-R-mephobarbital and (-)-R-mephenytoin individually under the same conditions. Pure enantiomers of benzonal were not available. Thus, the peak that eluted at a lower capacity factor was identified as (+) S-mephobarbital while the (-)-R-enantiomer eluted at a higher capacity factor. The elution order was reversed in the case of mephenytoin, as the (-)-R-mephenytoin eluted with a lower capacity factor followed by the (+)-S-enantiomer. The chromatographic conditions used to resolve the three antiepileptic drugs under study are summarized in Table 1.

RESULTS AND DISCUSSION

The separation of three antiepileptic drugs commonly administered clinically as the racemate mixture has been achieved using Chiralcel OJ column. A chromatogram of the enantiomeric separation of mephobarbital, mephenytoin and benzonal are shown in Figure 2 A, B, and C, respectively.

This cellulose-based chiral stationary phase has been used successfully to separate directly several barbiturates (28) and other structurally related drugs such as glutethimide (29), succinimides e.g. methsuximide and hydantoins e.g. ethotoin (30).

It is of interest to mention, however, that a reversal of enantiomeric elution order of mephenytoin enantiomers was observed when compared to that of mephobarbital enantiomers, i.e. the (-)-R mephenytoin eluted first followed by the (+)-S-enantiomer (as shown in Fig. A and B, respectively). Mephobarbital and mephenytoin are structurally related compounds except for the fact that mephobarbital is a six-membered heterocyclic ring, namely, 2,4,6-pyrimidinetrione while mephenytoin is a five-membered heterocyclic ring, namely, 2,4-imidazolidinedione. Actually, in terms of real spatial arrangements of C-5 substituents against the ring this for (+)-S- mephobarbital is virtually the same like that for

Table 1. Chromatographic Conditions and Parameters for Enantioselective Separation of Some Antiepileptic Drugs

Compound	Mobile phase	Flow rate ml/min	Sample amount for racemic mixture injected in nmoles	$'k_1$	$'k_2$	α	R_s
Mephobarbital	Methanol (100%)	1.0	21	1.28 (+)S	3.88 (-)-R	3.03	3.81
Mephenytoin	Methanol (100%)	0.5	20	0.86 (-)R	1.25 (+)-S	1.46	0.73
Benzonal	Ethanol:methanol (90:10)	0.8	10.6	3.75	4.53	1.21	0.54

$'k_1$ = capacity factor for 1st eluted enantiomer
$'k_2$ = capacity factor for 2nd eluted enantiomer
α = stereochemical separation factor or selectivity
R_s = stereochemical resolution factor
Temperature maintained at 23°C and chart speed was 0.5 cm/min

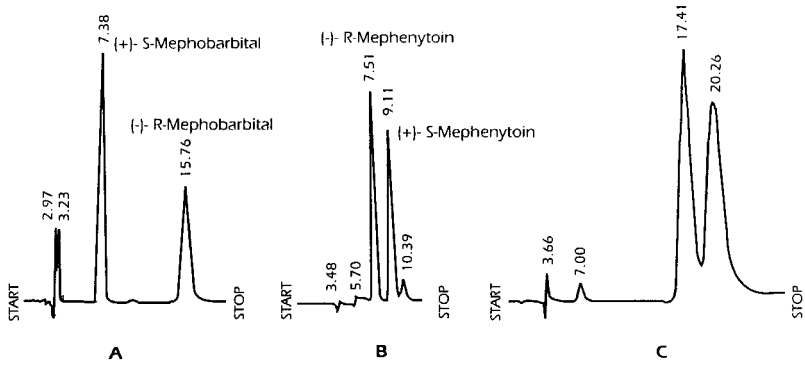

Figure 2. A. LC separation of RS-Mephobarbital enantiomers.
B. LC separation of RS-Mephenytoin enantiomers.
C. LC separation of RS-Benzonal enantiomers.
Chromatographic conditions are described in Experimental and Table 1.

(-)-R- mephenytoin and vice versa. This is confirmed by the analysis of the absolute configurations of I and II according to Cahn, Ingold and Prelog rules. The lack of one carbonyl group in mephenytoin and the presence of the methyl group at C-3 makes this difference. Accordingly, one would expect that the same binding points for (+)-S-mephobarbital and (-)-R-mephenytoin to the CSP would be the same. Thus, the ring contraction of II to the five-membered hydantoin ring and slight chemical changes between I and II caused (+)-S-mephobarbital and (-)-R-mephenytoin to be eluted first under the same chromatographic conditions.

However, based on the chemical structure-chiral recognition relationships of the same class of compounds, one can assume that in the case of benzonal, the (+)-S-enantiomer is expected to elute first. This rationale is based on the fact that the enantiomeric separation depends on the hydrogen bonding between the imidic-NH-group of the drug and the carbonyl ester group of the cellulose 4-methylbenzoate derivative, which is essential for effective chiral recognition. Dipole-dipole interactions, partial inclusion and the geometry of the enantiomer in the CSP also play an important role for the stereochemical separation (26,30). The stereochemical factor (R_S) of benzonal was only 0.54 with partial enantiomeric resolution (Fig 2C) contrary to mephobarbital and mephenytoin. This may be due to the presence of the benzoyl group in benzonal which increases the lipophilicity of the compound (31) and accordingly the strength of interactions with the CSP.

This method can be applied for enantiomeric purity determinations of these drugs in bulk and pharmaceutical dosage forms. Also, the simplicity of this method permits its application in determination of phenotype frequencies in patients receiving mephenytoin and mephobarbital as poor or extensive metabolizers which is currently in progress. The data generated from this study will allow to avoid adverse reactions to drug medications. Furthermore, this column in preparative form could be used to separate large quantities of individual enantiomers of the respective drugs for further pharmacodynamic and phamacokinetic evaluation.

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