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Note

Some structural requirements for resolution of hydantoin enantiomers with a β-cyclodextrin liquid chromatography column

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Covalently-linked β-cyclodextrins have been developed as high-performance liquid chromatography (HPLC) chiral stationary phases for the resolution of enantiomers, as has been recently described[1–3]. The geometry of the β-cyclodextrin (cycloheptaamylose) molecule is that of a hollow, truncated cone with the interior cavity being hydrophobic. The secondary and primary alcohols of the constituent glucose units are located on the edges of the the wider and narrower openings of the cone, respectively. Recent studies of the interaction of selected enantiomers with β-cyclodextrin suggested that, in addition to the hydrophobic binding site, successful resolution requires interactions of the secondary alcohols of the β-cyclodextrin with substituents on the compound being resolved[2].

Studies in this laboratory have demonstrated the utility of β-cyclodextrin columns (Cyclobond I, Astec, Whippany, NJ, U.S.A.) in the resolution of phenolic metabolite enantiomers of 5,5-diphenylhydantoin[4]. A project to continue studies of the stereoselective metabolism of 5-phenylhydantoins required methodology for the resolution of a variety of substituted hydantoins. This report describes the ability of β-cyclodextrin HPLC columns to resolve some selected substituted hydantoins, and how such studies have offered insights into the mechanisms of resolution with these columns.

EXPERIMENTAL

Instrumentation

A gradient chromatograph was composed of two Altex Model 110A pumps (Berkeley, CA, U.S.A.) controlled by an Axxiom Model 710 microprocessor. A Rheodyne Model 7125 injector with a 20-μl sample loop (Cotati, CA, U.S.A.) and an ISCO Model V4 variable-wavelength detector (Lincoln, NE, U.S.A.) (set at 254 nm) were coupled to the system. Chromatographic peaks were integrated by use of a Hewlett-Packard Model 3390A reporting integrator (Avondale, PA, U.S.A.).

Columns and temperature control

β-Cyclodextrin and γ-cyclodextrin HPLC columns (5 μm, 250 × 4.6 mm I.D.) were purchased from Astec, and were coupled to the gradient chromatograph with

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a 0.5-μm in-line filter. Temperature control was achieved at 22°C with the use of a water jacket and a thermostatted circulating water bath. Column flow-rates were set at 0.8 ml/min. Optimal flow-rates for individual compounds were not investigated.

Chemicals

HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Pittssburgh, PA, U.S.A.). HPLC-grade water was produced from distilled deionized water that had been treated with a Millipore Norganic filter system from Waters Assoc. (Milford, MA, U.S.A.).

5-Alkyl-5-phenylhydantoins (compounds 2–6) were prepared from the corresponding alkyl phenyl ketones via the method of Sobotka *et al.*[5]. A sample of 5-phenylhydantoin (1) was graciously provided by Dr. Kenneth H. Dudley (University of North Carolina, Chapel Hill, NC, U.S.A.). Enantiomers of 5-ethyl-5-phenylhydantoin (3) were obtained from resolution of the brucine salts according to the method of Sobotka *et al.*[5].

3-Alkyl-5-ethyl-5-phenylhydantoins (7–14) were prepared from racemic 5-ethyl-5-phenylhydantoin (3) by alkylation with the appropriate alkyliodides as described by Maguire *et al.*[6]. Enantiomers of 5-ethyl-3-methyl-5-phenylhydantoin (7) were prepared from enantiomers of 5-ethyl-5-phenylhydantoin (3) by the method described in ref. 7.

5-Ethyl-5-(4-hydroxyphenyl)hydantoin (16) and its enantiomers were available from a previous study by Butler[8] as were 5-ethyl-3-methyl-5-(4-hydroxyphenyl)hydantoin (15) and its enantiomers[9], and 5-(4-hydroxyphenyl)-5-(1-propyl)hydantoin (17)[10]. 5-(4-Methylphenyl)-5-phenylhydantoin (18) and 5-(4-hydroxyphenyl)-5-(4-methylphenyl)hydantoin (19) were purchased from Aldrich (Milwaukee, WI, U.S.A.).

All compounds were prepared as methanolic solutions at a concentration of 1.0 mg/ml.

RESULTS

5-Alkyl-5-phenylhydantoins

In the series of six 5-alkyl-5-phenylhydantoins (Fig. 1), two trends can be observed in the chromatographic properties as examined on a β-cyclodextrin HPLC column (Table I). With methanol–water eluents the effect of increasing the alkyl substituent is generally to increase the retention times, for substituents up to *n*-propyl and isopropyl. A comparison of the chromatographic properties of the isomeric propyl compounds is shown in Fig. 2. It would appear that, for *n*-butyl, and perhaps larger alkyl substituents, the increasing retention time pattern may not hold, as the 5-(1-butyl)-5-phenylhydantoin (6) has a smaller capacity factor (k') and less resolution (R_s) than that of either the *n*-propyl or the isopropyl derivatives. The correlation of increasing retention times with increasing lengths of alkyl groups would be consistent with a hydrophobic interaction of the 5-alkyl group with the interior, hydrophobic cone of the β-cyclodextrin. Examination of Corey–Pauling–Koltun (CPK) molecular models does suggest that an optimal size of 5-alkyl substituent allows for a tighter "fit" of the phenyl substituent into the hydrophobic cavity, and a potentially increased enantiomeric discrimination (Table I). The presence of *n*-butyl and larger

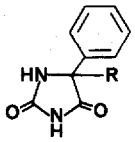

Fig. 1. The chemical structure of the 5-alkyl-5-phenylhydantoins described in Table I.

TABLE I

RESOLUTION OF 5-ALKYL-5-PHENYLHYDANTOIN ENANTIOMERS ON A β-CYCLODEXTRIN HPLC COLUMN

Compound		*10% Methanol*			*20% Methanol*			*10% Acetonitrile*		
No.	*R**	*k'***	α	R_s	*k'***	α	R_s	*k'***	α	R_s
1	H	0.67	1.09	0.2	0.33	1.00	0	0.23	1.00	0
2	CH_3	1.17	1.11	0.5	0.76	1.00	0	0.64	1.00	0
3	C_2H_5	2.30***	1.21	2.0	1.38***	1.17	1.3	1.59***	1.20	2.0
4	1-C_3H_7	3.30	1.26	2.2	1.76	1.22	1.8	1.09	1.38	2.0
5	2-C_3H_7	4.96	1.27	2.5	2.48	1.25	2.2	2.45	1.32	2.4
6	1-C_4H_9	4.40	1.14	1.1	2.24	1.11	0.8	2.32	1.18	1.1

* Substituent indicated on the structure shown in Fig. 1.
** *k'* values are those of the first-eluting enantiomer.
*** (*R*)-(−)-enantiomer eluted first.

substituents on CPK models suggests that these may interfere with possible hydrogen-bonding of the hydantoin ring functionality to the secondary hydroxyl groups of the β-cyclodextrin.

The chromatographic properties of this series of compounds were compared with a 10% acetonitrile eluent (Table I), and were found to display a similar pattern of retention time and resolution, with the exception of the 1-propyl substituent (4). In fact, the *k'*, α (separation factor) and R_s values observed for compounds 1–6 for 20% methanol are remarkably similar to those observed in 10% acetonitrile. Examination of the chromatographic properties of compounds 1–6 on a γ-cyclodextrin column (methanol or acetonitrile–water eluents) gave no resolution of compounds, with the exception of partial resolution ($R_s < 0.5$) of the isopropyl compound (5) (data not shown).

3-Alkyl-5-ethyl-5-phenylhydantoins

In contrast to the behavior of 5-alkyl-5-phenylhydantoins, the effect of increasing the 3-alkyl substituent on the chromatographic properties of compounds 3, and 7–14 was not noticible (Fig. 3, Table II). For the first four members of the the series of *n*-alkyl substituents (compounds 3, 7, 8, and 9), there is no obvious trend in the *k'* or R_s values as hydrophobicity increases, suggesting an alternative mechanism of enantiomeric discrimination. If one examines the *n*-alkyl compounds (3, 7–9, 11, and 13), maximal resolution is achieved with the methyl substituent (7), and further elongation of the chain results in decreasing R_s values. Examination of CPK models of the *n*-alkyl derivatives suggested that the larger alkyl substituents interfered with the ability of the hydantoins to hydrogen bond with the secondary hydroxyl groups on the β-cyclodextrin. This hypothesis was further tested by synthesizing the isopropyl

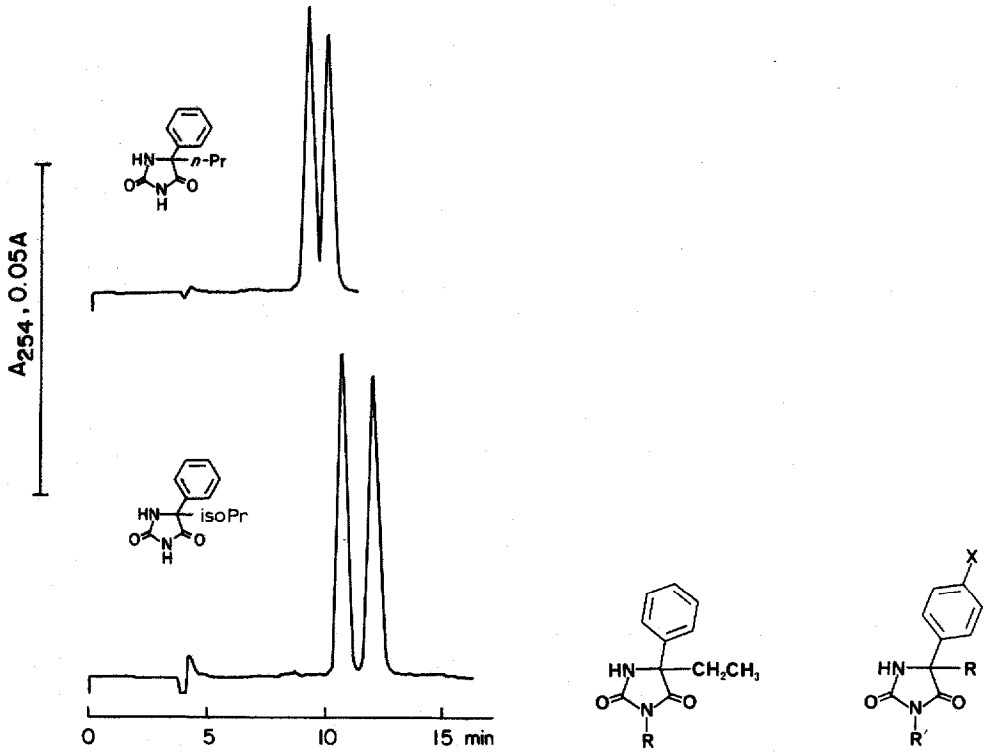

Fig. 2. Chromatograms of the enantiomeric resolution of 5-(isopropyl)- and 5-(*n*-propyl)-5-phenylhydantoins on a β-cyclodextrin HPLC column with 20% methanol as eluent. Pr = Propyl.

Fig. 3. The chemical structure of the 3-alkyl-5-ethyl-5-phenylhydantoins described in Table II.

Fig. 4. The chemical structure of the 3-alkyl-5-(4-X-phenyl)hydantoins and related compounds as described in Table III.

TABLE II

RESOLUTION OF 3-ALKYL-5-ETHYL-5-PHENYLHYDANTOIN ENANTIOMERS ON A β-CYCLODEXTRIN HPLC COLUMN

Compound		*10% Methanol*			*20% Methanol*		
No.	*R**	*k'***	α	R_s	*k'***	α	R_s
3	H	2.30***	1.21	2.0	1.38***	1.17	1.3
7	CH_3	2.15***	1.47	4.2	1.00***	1.48	2.3
8	C_2H_5	2.18	1.26	2.2	1.05	1.22	1.5
9	1-C_3H_7	3.13	1.13	1.2	1.50	1.13	0.8
10	2-C_3H_7	2.88	1.00	0	1.20	1.00	0
11	1-C_4H_9	5.28	1.08	0.4	2.60	1.04	0.2
12	iso-C_4H_9	6.38	1.00	0	2.85	1.00	0
13	1-C_5H_{11}	14.3	1.00	0	6.25	1.00	0
14	$CH_2C_6H_5$	17.8	1.00	0	9.70	1.00	0

* Substituent indicated on the structure shown in Fig. 3.
** *k'* values are those of the first-eluting enantiomer.
*** (*R*)-(−)-enantiomer elutes first.

TABLE III

RESOLUTION OF 3-ALKYL-5-SUBSTITUTED-5-(4-X-PHENYL)HYDANTOIN ENANTIOMERS ON A β-CYCLODEXTRIN HPLC COLUMN

Compound No.	*Substituent**			*10% Methanol*			*20% Methanol*		
	R	*R'*	*X*	*k'***	*α*	*R_s*	*k'***	*α*	*R_s*
7	C_2H_5	CH_3	H	2.15***	1.47	4.2	1.00***	1.48	2.3
15	C_2H_5	CH_3	OH	2.48***	1.49	4.7	1.06***	1.47	3.6
3	C_2H_5	H	H	2.30***	1.21	2.0	1.38***	1.17	1.3
16	C_2H_5	H	OH	2.87***	1.22	2.3	1.15***	1.20	1.6
4	C_3H_7	H	H	3.30	1.26	2.2	1.76	1.22	1.3
17	C_3H_7	H	OH	5.98	1.31	3.6	2.75	1.29	2.2
18	$CH_3C_6H_4$	H	H	N.D.§	N.D.	N.D.	10.9	1.11	1.0
19	$CH_3C_6H_4$	H	OH	N.D.	N.D.	N.D.	14.7	1.16	1.4

* Substituents as indicated on the structure shown in Fig. 4.
** *k'* values are those of the first-eluting enantiomer.
*** (*R*)-(−)-enantiomer elutes first.
§ N.D., not determined.

(10) and isobutyl (12) compounds and comparing their chromatographic properties. These two compounds were not resolved by the β-cyclodextrin column. This evidence is consistent with the suggestion that the disruption of hydrogen bonding by bulky 3-substituents does not allow enantiomeric discrimination.

p-Hydroxyphenylhydantoins

An appropriate supply of variously substituted 5-(4-hydroxyphenyl)-hydantoins (Fig. 4) allowed further study of the structural requirements for optimal resolution of selected hydantoins (Table III). In all the pairs of compounds shown in Table III, the *p-* or 4-hydroxyphenyl derivatives (15–17, and 19) would be considered to be more hydrophilic than the corresponding unsubstituted compounds (7, 3, 4, and 18). Yet in all cases, the k' and R_s values are greater for the 4-substituted compounds. One appropriate conclusion is that the 4-hydroxy derivatives offer an additional site of interaction with the β-cyclodextrin moiety, other than the hydrophobic site. Examination of CPK models of 4-hydroxyphenyl derivatives complexing with β-cyclodextrin indicate that the phenolic substituent can complex, via hydrogen bonding, with the primary hydroxyl groups at the edge of the narrower end of the β-cyclodextrin molecule. This would be consistent in all cases with the increased k' and R_s values of the 4-hydroxyphenyl derivatives as compared to the phenyl-substituted compounds.

DISCUSSION

Assuming that the resolution of 5-phenylhydantoins by β-cyclodextrin columns is a result of the phenyl substituent binding in the hydrophobic interior of the cyclodextrin, the successful resolution of enantiomers requires at least two additional sites of interaction. In the case of the 5-alkyl-5-phenylhydantoins (Table I, Fig. 1), there appears to be an optimal size for the 5-alkyl substituent in assisting the hydrophobic binding of the molecules. With such direction of the molecules in the β-cy-

clodextrin, the carbonyl, imide, and amide functionalities of the hydantoin ring present themselves in such a manner as to allow at least two hydrogen bonds with the β-cyclodextrin secondary hydroxyl groups. These three sites would then allow for enantiomeric discrimination by the column.

Further evidence for the importance of the hydrogen bonds with the hydantoins was obtained by investigating the effects of the 3-alkyl substituent on resolution (Table II, Fig. 3). When sufficiently large groups are introduced at the 3-position, hydrogen bonding is disrupted, and enantiomeric discrimination is eliminated. All of this experimental evidence is consistent with the conclusions of Armstrong *et al.*[2] as to the mechanisms of enantiomeric discrimination.

The increased k' and R_s values for the 4-hydroxyphenyl-substituted hydantoins compared to the unsubstituted compounds (Table III, Fig. 4) offers the first evidence that hydrogen bonding of *p*-phenols to the primary hydroxyl groups of the β-cyclodextrin may be of importance in increasing resolution. In all of the pairs of compounds shown in Table III, increased k' and R_s values would be consistent with the existence of such hydrogen bonding.

In addition to the knowledge of enantiomeric discrimination by use of β-cyclodextrin HPLC columns, the practical application of such knowledge to analytical challenges has proved possible. Specifically, as compounds 3, 7, 15, and 16 all are resolved to better than baseline resolution (Table III), it has been possible to develop HPLC assays for determining the enantiomeric content of such urinary metabolites of mephenytoin (7)[11]. This is especially important in the case of monitoring patients treated with mephenytoin (7), an antiepileptic drug that has been shown to be stereoselectively metabolized[12] via hydroxylation to 15, and demethylation to an active antiepileptic compound (3), which is further metabolized to its *p*-hydroxy metabolite (16).

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