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APPLICATION OF α- AND β-CYCLODEXTRIN AND HEPTAKIS(2,6-DI-O-METHYL)-β-CYCLODEXTRIN AS MOBILE PHASE COMPONENTS FOR THE SEPARATION OF SOME CHIRAL BARBITURATES INTO ENANTIOMERS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Using LiChrosorb RP-18 as the stationary phase, systematic studies were performed on the changes in the capacity factors of a series of hydantoin and barbituric acid derivatives (including some therapeutically useful drugs) with variation in the concentrations of α- and β-cyclodextrin and heptakis(2,6-di-O-methyl)-β-cyclodextrin in the mobile phase.It has been found that both β-cyclodextrin and its dimethyl derivative exhibit enantioselectivities towards hydantoins and barbiturates that contain a chiral carbon atom in the heterocyclic ring. No selectivity was observed with α-cyclodextrin. Owing to the strong adsorption of dimethylated β-cyclodextrin from its dilute solutions on an ODS surface, a new mechanism for the separation of enantiomeric barbiturates was established.

INTRODUCTION

The remarkable ability of cyclodextrins (CDs) to include selectively in their cavities many chemical species is well known[1]. This property has been taken advantage of in many separation techniques, including various types of chromatography[2,3].

The utilization of CD inclusion processes in high-performance liquid chromatography (HPLC) has been carried out by two different approaches. The first relies on the use of CDs chemically bonded to silica phases via the nitrogen atom[4–6] or by a spacer containing no nitrogen or sulphur linkage[7]. The latter type of packing is stable towards hydrolysis and as it is commercially available it can be used as a powerful tool for the separation of structural isomers[8,9] and chiral compounds[7,9–11], including some chiral barbiturates[7,10].

In the second approach CDs applied as mobile phase components in order to impart their selective complexation properties to reversed-phase (RP) system[12–19].

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Using this approach we have recently successfully resolved mephenytoin and two barbiturates (hexobarbital and methylphenobarbital) into their enantiomers[18].

Further development of this procedure for the resolution of chiral compounds of the same groups was attempted in this work using α-CD, β-CD and heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD). RP systems modified with CD are more complicated than those with CD stationary phases; two factors influence their resolving power, namely complexation and adsorption. On the other hand, these phenomena may often be advantageous for the control and adjustment of the separation process.

EXPERIMENTAL

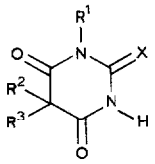
Reagents

β-CD was supplied by Chinoin (Budapest, Hungary) and α-CD and DM-β-CD were kindly provided by Prof. J. Szejtli (Chinoin). All solvents and reagents were of analytical-reagent grade and were used without purification.

The formulae of the investigated solutes are given in Table I. Most of them are well known commercially available drug products.

TABLE I

STRUCTURAL FORMULAE OF THE INVESTIGATED COMPOUNDS



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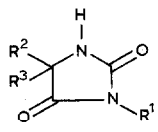

8,9

Compound	*X*	R^1	R^2	R^3	*Name (manufacturer)*
1	O	H	1-Methylbutyl	Ethyl	Pentobarbital (Abbott)
2	O	H	1-Methylbutyl	Allyl	Secobarbital (Rhone-Poulenc)
3	S	H	1-Methylbutyl	Ethyl	Thiopental (Abbott)
4	O	CH_3	Phenyl	Ethyl	Methylphenobarbital (Polfa)
5	O	CH_3	Cyclohexen-1-yl	Methyl	Hexobarbital (Polfa)
6	O	CH_3	Cyclohexyl	Ethyl	
7	O	CH_3	Phenyl	Allyl	
8		H	Phenyl	Ethyl	
9		CH_3	Phenyl	Ethyl	Mephenytoin (Alkaloida)

Pure enantiomers of 4 and 7 were kindly provided by Prof. J. Knabe (Saarbrücken, F.R.G.) and compounds 6 and 8 were donated by Prof. G. Blaschke (Münster, F.R.G.) and Prof. B. Gutkowska (Warsaw, Poland), respectively.

The enantiomers of 5 and 9 were prepared by means of micro-preparative chromatography using β-CD solution as the mobile phase according to the method described earlier[18]. The configuration of mephenytoin enantiomers was established according to ref. 20.

Apparatus and procedures

Two types of HPLC apparatus, 302 and 310 (Institute of Physical Chemistry, Warsaw, Poland), were used, equipped with a UV detector (254 nm) containing 10-μl and 0.4-μl flow cells and injectors with volumes of 5 μl and 0.4 μl, respectively.

Experiments were carried out with columns of dimensions 250 × 4.0 mm I.D. and 100 × 4.0 mm I.D. packed with 10-μm LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.) and 250 × 1.0 mmmmm I.D. packed with 5-μm LiChrosorb RP-18.

The mobile phases were ethanol–phosphate buffer pH 2 (20:80, v/v) solutions[18] containing various amounts of appropriate CDs.

The solutes injected onto the columns were dissolved in ethanol at a concentration of 1 m*M*.

RESULTS AND DISCUSSION

Influence of α- and β-CD and DM-β-CD on the separation process

The data in Table II demonstrate the influence of α- and β-CD and DM-β-CD on the resolution of some barbiturates and mephenytoin in the RP system. This comparison shows that not only β-CD[18] but also its dimethyl derivative exhibit enantioselectivity towards hydantoins and barbiturates that contain a chiral centre in the heterocyclic ring. In contrast, no distinguishable enantioselectivity was observed for α-CD complexation of barbiturates and hydantoin derivatives.

TABLE II

COMPARISON OF CAPACITY FACTORS (k') AND SELECTIVITY FACTORS (α) ON ODS COLUMNS WITH DIFFERENT CYCLODEXTRINS IN THE MOBILE PHASE

k'_G, k' = capacity factors for free molecules and first eluted peaks, respectively.

Compound	*25 mM α-CD**			*20 mM β-CD***			*25 mM DM-β-CD****		
	k'_G	k'	α	k'_G	k'	α	k'_G	k'	α
1	39.5	28.1	1.00	36.9	7.5	1.00			
2	60.1	44.1	1.00	58.2	9.4	1.00			
3	74.3	57.7	1.00	68.0	10.2	1.00			
4	21.1	17.9	1.00	20.9	5.0	1.08	18.9	4.3	1.07
5	19.3	15.5	1.00	18.1	4.0	1.07	16.5	5.2	1.02
9	18.5	13.7	1.00	15.0	8.4	1.15	13.7	5.1	1.21

* Column, 250 × 4.0 mm I.D.

** Columns, 250 × 4.0 mm I.D. for compounds 1–3 and 100 × 4.0 mm I.D. for 4, 5 and 9.

*** Column, 250 × 1.0 mm I.D.

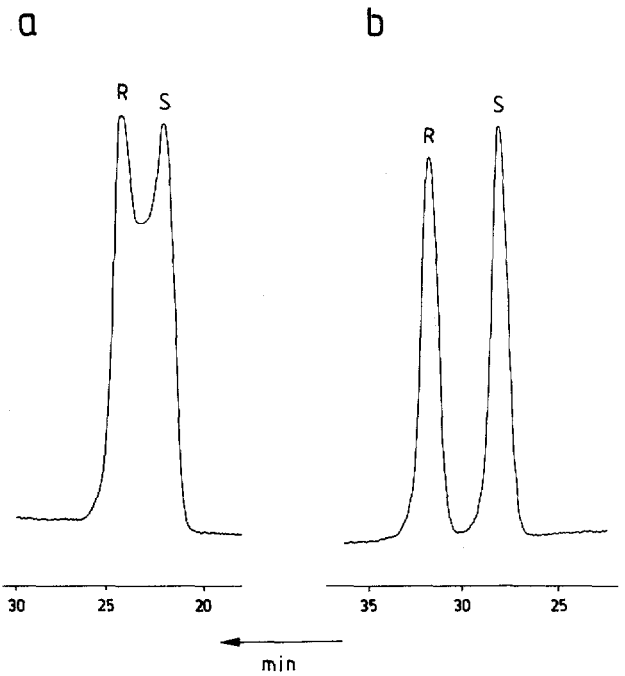

Fig. 1. Resolution of mephenytoin enantiomers on a 5-μm LiChrosorb RP-18 column (250 × 1.0 mm I.D.) with the mobile phases (a) 25 m*M* DM-β-CD ($\alpha_{R/S}$ = 1.21) and (b) 25 m*M* β-CD ($\alpha_{R/S}$ = 1.15) in ethanol–buffer (pH 2.0) (20:80, v/v). Flow-rate, 30 μl/min. Temperature, 22°C.

The use of DM-β-CD at a high concentration (25 m*M*) caused a decrease in column efficiency. For this reason, in spite of the large selectivity factor for mephenytoin (α = 1.21) in DM-β-CD solution, this compound can be only partially resolved into enantiomers, whereas the same solute is easily separated using β-CD at lower selectivity (α = 1.15), when the column efficiency is greater (Fig. 1).

Fig. 2 shows further examples of separations achieved using β-CD solution and performed on the microbore column.

Special effect of DM-β-CD

The addition of α-CD or β-CD to a mobile phase in an RP system is always followed by a decrease in capacity factor (*k'*). This behaviour was observed for the investigated compounds; another effect appears in dilute DM-β-CD solutions.

Fig. 3 shows examples of plots of *k'* values measured on the RP column *versus* DM-β-CD concentration for barbiturates and mephenytoin. The retention of all barbiturates are greater at low concentration of DM-β-CD than those determined without any additives in the mobile phases. Moreover, the order of elution of enantiomeric pairs of compounds 4, 5 and 7 changes with increasing concentration of DM-β-CD. In the concentration range 0.0–1.0 m*M*, when the retention values are at a maximum, the elution order of barbiturate enantiomers (4,5,7) is opposite to that observed in the β-CD solutions. When the concentration of DM-β-CD increases the elution order becomes the same as that in the β-CD solution, *i.e.*, the first eluted

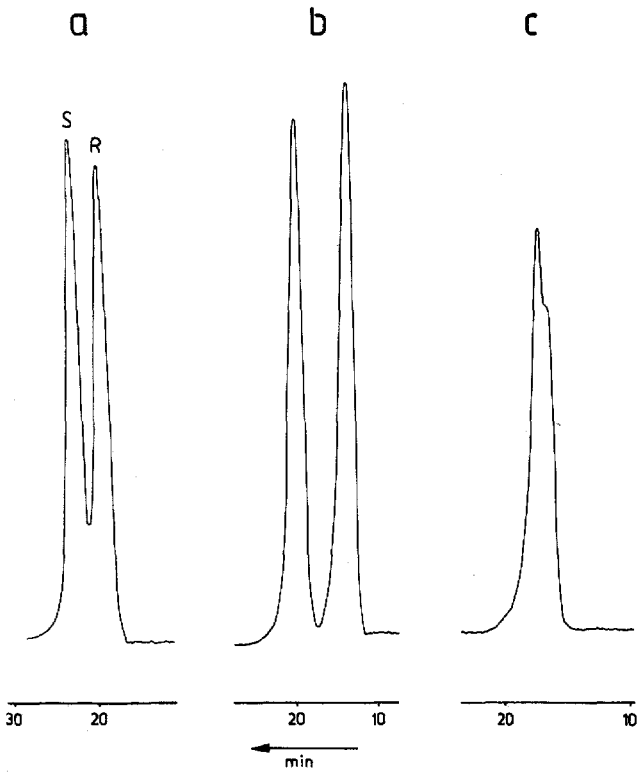

Fig. 2. Chromatograms of a racemic mixture of compounds (a) 4, (b) 6 and (c) 8 obtained on a 5-μm LiChrosorb RP-18 column (250 × 1.0 mm I.D.) with 25 m*M* β-CD in ethanol–buffer (pH 2.0) (20:80, v/v). Flow-rate, 30 μl/min. Temperature, 22°C.

enantiomer has an *R* configuration. This phenomenon is exemplified in Fig. 4, demonstrating the resolutions of hexobarbital enantiomers performed with 25 m*M* β-CD and 0.8 m*M* and 25 m*M* DM-β-CD in ethanol–buffer (20:80).

An exception to this behaviour is shown by mephenytoin enantiomers, which leave the column in the same order (*S* first) when both β-CD and DM-β-CD solutions are used over the full range of concentrations. This confirms our earlier suggestion that the resolution mechanism for mephenytoin and barbiturates is different[18].

A similar increase in retention following small additions of methylated CDs to the mobile phase of an RP system was observed by Tanaka *et al.*[21] for disubstituted benzene derivatives. They suggested that this behaviour results from the strong adsorption of methylated CDs on the ODS stationary phase, which is due to their greater hydrophobic character than β-CD. Our results are in full agreement with this suggestion.

The inversion of selectivity observed with DM-β-CD solution, as exemplified in Figs. 3 and 4 and Table III, may be explained by assuming strong adsorption of this CD on the ODS stationary phase. In dilute solution the resolution of enantiomers is governed mainly by the processes of adsorption (inclusion) of the free molecules of the solute on the DM-β-CD layer adsorbed on the surface of the stationary phase;

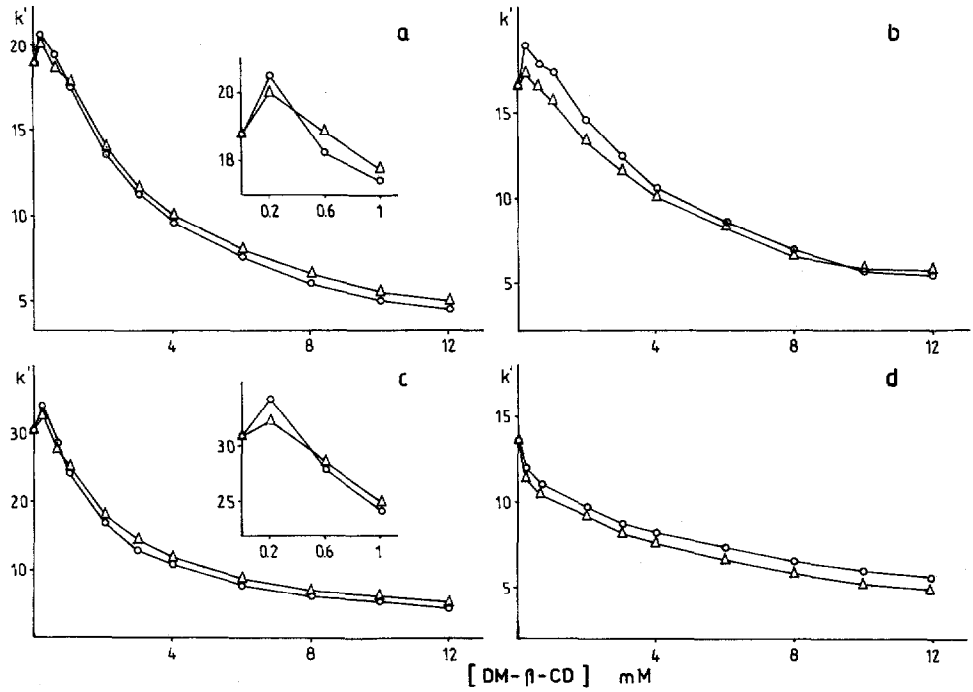

Fig. 3. Plots of capacity factors (k') of enantiomeric pairs (○, *R*; △, *S*) of compounds (a) 4, (b) 5, (c) 7 and (d) 9 *versus* DM-β-CD concentration. Stationary phase, 10-μm LiChrosorb RP-18. Temperature, 25°C.

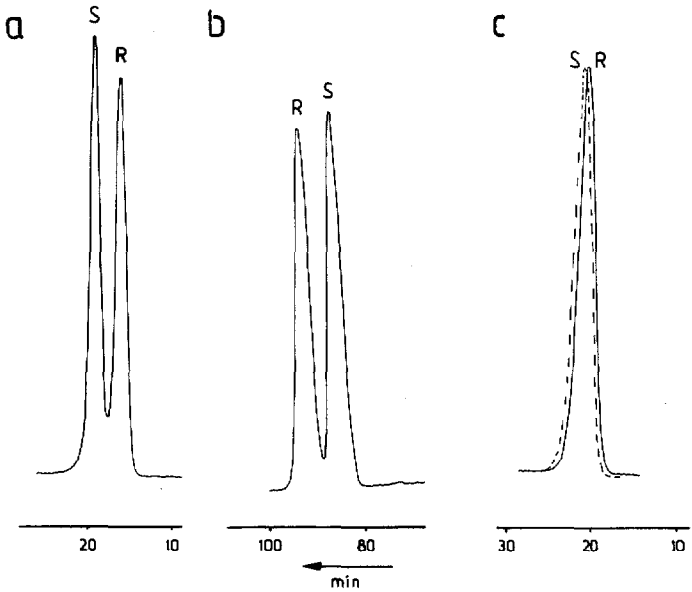

Fig. 4. Chromatograms of hexobarbital enantiomers obtained on a 5-μm LiChrosorb RP-18 column (250 × 1.0 mm I.D.) with (a) 25 m*M* β-CD, (b) 0.8 m*M* DM-β-CD and (c) 25 m*M* DM-β-CD in ethanol–phosphate buffer pH 2 (20:80, v/v). Flow-rate, 30 μl/min. Temperature, 22°C. Chromatogram (b) was obtained after dynamic equilibration of the column by passing *ca.* 30 ml of the mobile phase solution.

TABLE III

INFLUENCE OF β-CD AND DM-β-CD CONCENTRATIONS IN THE MOBILE PHASE ON SEPARATION FACTORS FOR BARBITURATES AND MEPHENYTOIN

Compound	*[β-CD] (mM)*						
	1.0	*2.0*	*4.0*	*8.0*	*12.0*	*25.0*	
4	1.01	1.03	1.04	1.07	1.07	1.08	
5	1.02	1.03	1.06	1.08	1.10	1.11	
9	1.01	1.02	1.04	1.07	1.10	1.16	
	[DM-β-CD] (mM)						
	0.2	*0.6*	*1.0*	*2.0*	*4.0*	*8.0*	*12.0*
4	0.98	0.98	1.02	1.02	1.03	1.07	1.11
5	0.95	0.91	0.91	0.93	0.96	0.99	1.02
7	0.97	1.00	1.02	1.05	1.10	1.11	1.12
9	1.02	1.02	1.02	1.02	1.06	1.06	1.11

the contribution of inclusion in the bulk solution seems to be negligible, because the equilibrium is shifted in favour of the free molecules. With increasing concentration of DM-β-CD the influence of complexation in the solution increases, which results in a change in the elution order.

HPLC has been widely used for the measurement of the stability constants of ion pairs and complexes with crown ethers[22], cyclodextrin[23], etc. Previously[18] we used eqn. 1 for evaluation of stability constants of complexes with β-CD:

$$k' = \frac{k'_G + k'_{G \cdot CD} K[CD]}{1 + K[CD]} \quad (1)$$

where k'_G and $k'_{G \cdot CD}$ are the capacity factors of the solute (G) free and bound to the CD molecule, respectively, and K is the stability constant of the G · CD complex. A linear transformation, k' *vs.* $(k'_G - k')/[CD]$, was used for the calculation of K and $k'_{G \cdot CD}$ values.

Fig. 5 shows plots of k' *vs.* $(k'_G - k')/[CD]$ for both β-CD and DM-β-CD. The straight lines for the β-CD system gave $k'_{G \cdot CD}$ values slightly below zero. This may be caused by a weak adsorption of β-CD on the ODS surface. For methylated β-CD the straight lines could be drawn only in the DM-β-CD concentration range 4.0–12.0 m*M*. The intercepts are more negative than those for β-CD. The curves in Fig. 5b indicate that the chromatographic model with DM-β-CD cannot be described by eqn. 1. According to Horváth *et al.*[22], such deviations from eqn. 1 are due to the strong adsorption of the complexing agent on the stationary phase. This phenomenon makes the complexation possible not only in the mobile phase but also on the modified ODS stationary phase.

The new systems with chiral dynamically generated DM-β-CD stationary phase are under further study.

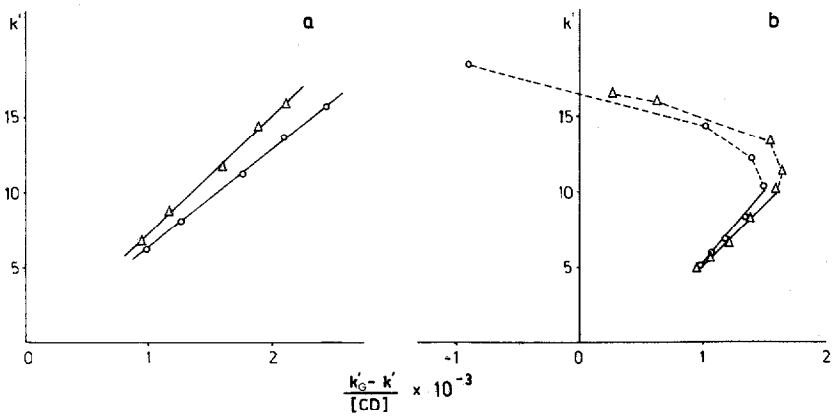

Fig. 5. Plots of k' *vs.* $(k'_G - k')/[CD]$ values for hexobarbital enantiomers (○, R; △, S) determined on a 10-μm LiChrosorb RP-18 column using (a) β-CD and (b) DM-β-CD in ethanol–buffer (20:80, v/v).

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REFERENCES

1 J. Szejtli, *Cyclodextrins and Their Inclusion Complexes*, Akadémiai Kiadó, Budapest, 1982.
2 W. L. Hinze, *Sep. Purif. Methods*, 10 (1981) 159.
3 E. Smolková-Keulemansová, *J. Chromatogr.*, 251 (1982) 17.
4 K. Fujimura, T. Ueda and T. Ando, *Anal. Chem.*, 55 (1983) 446.
5 Y. Kawaguchi, M. Tanaka, M. Nakae, K. Funazo and T. Shono, *Anal. Chem.*, 55 (1983) 1852.
6 M. Tanaka, Y. Kawaguchi, M. Nakae, Y. Mizobuchi and T. Shono, *J. Chromatogr.*, 299 (1984) 341.
7 D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci.*, 22 (1984) 411.
8 D. W. Armstrong, A. Alak, K. Bui, W. DeMond, T. Ward, T. E. Riehl and W. L. Hinze, *J. Incl. Phenom.*, 2 (1984) 533.
9 D. W. Armstrong, W. DeMond, A. Alak, W. L. Hinze, T. E. Riehl and K. H. Bui, *Anal. Chem.*, 57 (1985) 234.
10 W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. De Mond, A. Alak and T. Ward, *Anal. Chem.*, 57 (1985) 237.
11 D. W. Armstrong, W. DeMond and B. P. Czech, *Anal. Chem.*, 57 (1985) 481.
12 J. Dębowski, D. Sybilska and J. Jurczak, *J. Chromatogr.*, 237 (1982) 303.
13 D. Sybilska, J. Lipkowski and J. Wójcikowski, *J. Chromatogr.*, 253 (1982) 95.
14 J. Dębowski, D. Sybilska and J. Jurczak, *Chromatographia*, 16 (1982) 198.
15 J. Dębowski, J. Jurczak and D. Sybilska, *J. Chromatogr.*, 282 (1983) 83.
16 Y. Nobuhara, S. Hirano and Y. Nakanishi, *J. Chromatogr.*, 258 (1983) 276.
17 D. Sybilska, J. Dębowski, J. Jurczak and J. Żukowski, *J. Chromatogr.*, 286 (1984) 163.
18 D. Sybilska, J. Żukowski and J. Bojarski, *J. Liq. Chromatogr.*, 9 (1986) 591.
19 J. Żukowski, D. Sybilska and J. Jurczak, *Anal. Chem.*, 57 (1985) 2215.
20 H. J. Bartón, J. Bojarski and A. Żurowska, *Arch. Pharm. (Weinheim, Ger.)*, (1986) in press.
21 M. Tanaka, T. Miki and T. Shono, *J. Chromatogr.*, 330 (1985) 253.
22 Cs. Horváth, W. Melander and A. Nahum, *J. Chromatogr.*, 186 (1979) 371.
23 K. Uekama, F. Hirayama, S. Nasu, N. Matsuo and I. Irie, *Chem. Pharm. Bull.*, 26 (1978) 3477.