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RESOLUTION OF SOME CHIRAL MANDELIC ACID DERIVATIVES INTO ENANTIOMERS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY VIA α- AND β-CYCLODEXTRIN INCLUSION COMPLEXES

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SUMMARY

The resolution of mandelic acid derivatives (differing in the kind of functional group and in the position of its substitution in the aromatic ring) into enantiomers in a reversed-phase high-performance liquid chromatographic system via α- and β-cyclodextrin inclusion complexes was studied. Of the mandelic acid derivatives investigated (*o*-, *m*- and *p*-OH, *o*-CH_3, *o*-OCH_3, *o*- and *m*-Cl), only the chloro derivatives showed high enantioselectivity in the processes of complex formation with β-cyclodextrin (separation factors: $\alpha_{o\text{-Cl}} = 1.8$ at pH 2.1 and $\alpha_{o\text{-Cl}} = 1.15$ and $\alpha_{m\text{-Cl}} = 1.15$ at pH 6.8). In contrast, the enantioselectivity for complex formation between α-cyclodextrin and mandelic acid and the derivatives investigated was low.

INTRODUCTION

Cyclodextrins (CDs) are toridal-shaped cyclic oligosaccharides made up of α-1,4-linked D-glucopyranose units. They exhibit a high stereoselective ability to form inclusion compounds with a variety of molecules and ions. Attempts to take advantage of this phenomenon in gas and liquid chromatography have been made, with interesting results[1,2].

We have recently applied β-cyclodextrin as the mobile phase component for resolution, in reversed-phase chromatographic systems, of racemic mandelic acid[3] and some of its derivatives with various substituents in the side-chain[4]. We have found that the enantioselectivity arising from inclusion in β-CD molecules was substantial only for compounds containing, at the chiral carbon atom, an intact carboxylic group and another polar group (*e.g.*, OH, NH_2) able to form a hydrogen bond. It was additionally assumed that the insertion of a phenyl group in the central cavity of β-CD provides the third point of contact, indispensable for achieving enantioselectivity in a chromatographic system, according to the 'three points of attachment" concept of Dalgliesh[5].

This work was designed to provide further experimental evidence for the suggested third point of contact, and to elucidate the relationship between enantioselec-

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tivity and the structure of the inclusion complexes. For these purposes systematic studies on the resolution of mandelic acid derivatives (differing in the kind of functional group and in the position of its substitution in the aromatic ring) into enantiomers were carried out.

EXPERIMENTAL

Reagents

α- and β-CD were supplied by Chinoin (Budapest, Hungary). Racemic *o*-, *m*- and *p*-hydroxy-, *o*- and *m*-chloro-, *o*-methyl- and *o*-methoxymandelic acids were prepared according to the general procedure described earlier[6]. Resolution of racemic *o*- and *m*-chloromandelic acids was carried out by the standard procedure with optically acvtive α-phenylethylamine as the resolving agent[7]. All other materials were of analytical- or laboratory-reagent grade, and were used without further purification.

Apparatus and procedure

Chromatographic experiments were performed using a high-performance liquid chromatographic (HPLC) unit constructed at the Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland, equipped with a spectrophotometric detector (254 nm) having a Z-shaped passage (volume 8 μl). Use was made of stainless-steel columns (250 × 4.5 or 50 × 4.5 mm I.D.), slurry-packed at 435 kg/cm^2 by the balanced density technique (10-μm LiChrosorb RP-18; E. Merck, Darmstadt, F.R.G.). All measurements were carried out at 25°C. The mobile phases consisted of aqueous solutions containing various concentrations of α- and β-CD and suitable buffer components.

As the resolution of racemic mandelic acid derivatives into enantiomers is very time consuming, the following simplified procedure was applied:

(1) preliminary evaluation of enantioselectivity by comparing chromatograms of the racemate of a given mandelic acid derivative with chromatograms of racemic mandelic acid itself, obtained under the same conditions;

(2) resolution into enantiomers of racemates of some compounds of interest;

(3) systematic investigations of retention with the use of enantiomeric forms of the above-mentioned compounds.

RESULTS AND DISCUSSION

The capacity ratio (k') and selectivity factors (α), determined in mobile phase solutions containing various α- or β-CD concentrations at two different pH values, are given in Tables I and II.

In the systems used, the equilibria of the investigated mandelic acid derivatives are complicated because:

(1) for each mandelic acid derivative, in the mobile phase solution, on the assumption that only complexes with 1:1 stoichiometry are formed, there are at least two inclusion complexes with different stabilities, formed by the neutral (MH) and anionic (M^-) species:

$$MH + CD \rightleftarrows MH \cdot CD;\ K^0 = \frac{[MH \cdot CD]}{[MH]\,[CD]}$$

$$M^- + CD \rightleftarrows (M \cdot CD)^-;\ K^- = \frac{[(M \cdot CD)^-]}{[M^-]\,[CD]}$$

TABLE I

CAPACITY RATIOS (k') AND SELECTIVITY FACTORS (α) OF MANDELIC ACID AND ITS DERIVATIVES AT DIFFERENT β-CYCLODEXTRIN CONCENTRATIONS AND AT TWO DIFFERENT pH VALUES OF THE MOBILE PHASE SOLUTIONS

Compound	*Parameter*	*[β-CD] (M × 10⁻³)*					
		pH 2.1			*pH 6.8*		
		0.0	*4.7*	*14.4*	*0.0*	*4.7*	*14.4*
Mandelic acid	$k'(+)$	15.0	7.77	4.88	1.05	0.87	0.75
	$k'(-)$	15.0	7.13	4.52	1.05	0.86	0.74
	$k'(\pm)$	15.0	7.45	4.70	1.05	0.87	0.75
	$\alpha = \frac{k'(+)}{k'(-)}$	1.00	1.09	1.08	1.00	1.01	1.01
p-Hydroxy-mandelic acid	$k'(+)$	4.60	1.30	0.80	0.90	0.56	0.51
	$k'(-)$	4.60	1.30	0.80	0.90	0.56	0.51
	$\alpha = \frac{k'(+)}{k'(-)}$	1.00	1.00	1.00	1.00	1.00	1.00
m-Hydroxy-mandelic acid	$k'(\pm)$	8.30	2.40	1.40	1.50	0.86	0.75
	$\alpha^\star$	1.00	< 1.09	< 1.08	1.00		
o-Hydroxy-mandelic acid	$k'(\pm)$	15.5	7.40	4.60	12.75	5.60	3.50
	$\alpha^\star$	1.00	< 1.09	< 1.08	1.00		
o-Methyl-mandelic acid	$k'(\pm)$	36.05	15.05	10.24	2.40	1.50	1.14
	$\alpha^\star$	1.00	< 1.09	< 1.08	1.00		
o-Methoxy-mandelic acid	$k'(\pm)$	24.2	10.02	7.05	2.20	0.94	0.65
	$\alpha^\star$	1.00	< 1.09	< 1.08	1.00		
o-Chloro-mandelic acid	$k'(+)$			119	55	20.9	11.35
	$k'(-)$			210	55	21.7	13.03
	$k'(\pm)$				55	21.3	
	$\alpha = \frac{k'(-)}{k'(+)}$			1.8	1.00	1.04	1.15
m-Chloro-mandelic acid	$k'(+)$				165		49.4
	$k'(-)$				165		56.6
	$k'(\pm)$				165	64.8	
	$\alpha = \frac{k'(-)}{k'(+)}$				1.00		1.15

★ This value was evaluated from a comparison with the pattern of the resolution of racemic mandelic acid under the same conditions.

TABLE II

CAPACITY RATIOS (k') AND SELECTIVITY FACTORS (α) OF MANDELIC ACID AND ITS DERIVATIVES AT DIFFERENT α-CYCLODEXTRIN CONCENTRATIONS AND AT TWO DIFFERENT pH VALUES OF THE MOBILE PHASE SOLUTIONS

Compound	*Parameter*	*[α-CD] (M × 10⁻³)*				
		pH 2.1			*pH 6.8*	
		0.0	*5.0*	*10.0*	*0.0*	*10.0*
Mandelic acid	$k'(+)$	15.0	6.48	4.87	1.05	0.36
	$k'(-)$	15.0	6.43	4.81	1.05	0.35
	$\alpha = \frac{k'(+)}{k'(-)}$	1.00	1.01	1.01	1.00	1.02
p-Hydroxy-mandelic acid	$k'(+)$	3.05	1.00	0.73	0.50	0.15
	$k'(-)$	3.05	1.00	0.73	0.50	0.15
	$\alpha = \frac{k'(+)}{k'(-)}$	1.00	1.00	1.00	1.00	1.00
m-Hydroxy-mandelic acid	$k'(\pm)$	6.15	2.38	1.79	1.5	0.23
	α	1.00			1.00	
o-Hydroxy-mandelic acid	$k'(\pm)$	12.8	5.12	4.25	10.6	1.08
	α	1.00				
o-Methyl-mandelic acid	$k'(\pm)$	36.05	19.8	13.2	2.4	0.58
	α	1.00			1.00	
o-Methoxy-mandelic acid	$k'(\pm)$	24.2	11.9	7.87	2.2	0.45
	α	1.00			1.00	
o-Chloro-mandelic acid	$k'(+)$				55	7.02
	$k'(\pm)$				55	7.02
	$\alpha = \frac{k'(+)}{k'(-)}$				1.00	1.00

(2) each of the at least four species [MH, M^-, MH · CD, $(M \cdot CD)^-$] is characterized by specific adsorption on the reversed phase [k'_{MH}, k'_{M^-}, $k'_{MH \cdot CD}$, $k'_{(M \cdot CD)^-}$].

Hence the measured overall capacity ratios are complex functions of pH and CD concentrations[8].

The data in Tables I and II lead to the following conclusions. In acidic solutions (pH 2.1), substituted mandelic acids (*o*-, *m*- and *p*-OH, *o*-Me, *o*-OMe), compared with mandelic acid itself, exhibited lower enantioselectivity of β-CD complex formation ($\alpha < 1.08$). Probably the observed drop in enantioselectivity arose from changes in the geometric requirements of the insertion of differently substituted aromatic rings into the β-CD cavity. Surprisingly, with chloro-substituted mandelic acid, a strong opposite effect was observed, *i.e.* the enantioselectivity of β-CD complex formation was very high ($\alpha = 1.8$ for *o*-chloromandelic acid). It is stressed that the

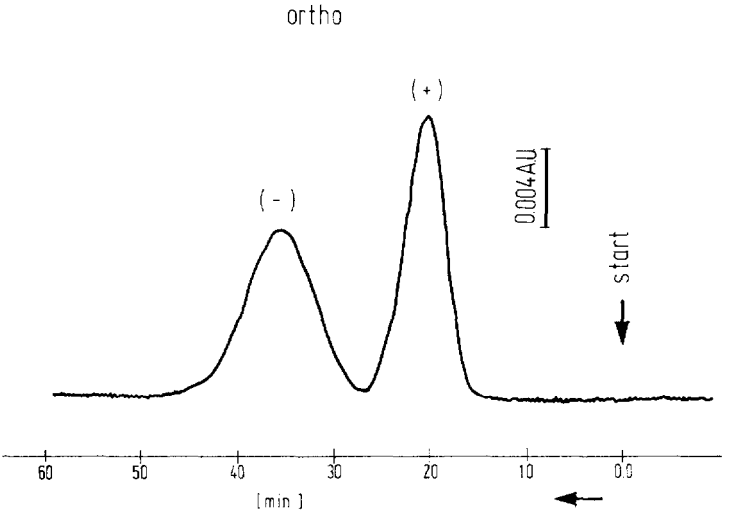

Fig. 1. Elution curve of racemic *o*-chloromandelic acid with an aqueous mobile phase of pH 2.1 and [*β*-CD] = $14.4 \cdot 10^{-3}$ *M*. Column, 50 × 4.5 mm I.D., LiChrosorb RP-18 (10 *μ*m); flow-rate, 2.4 ml/min.

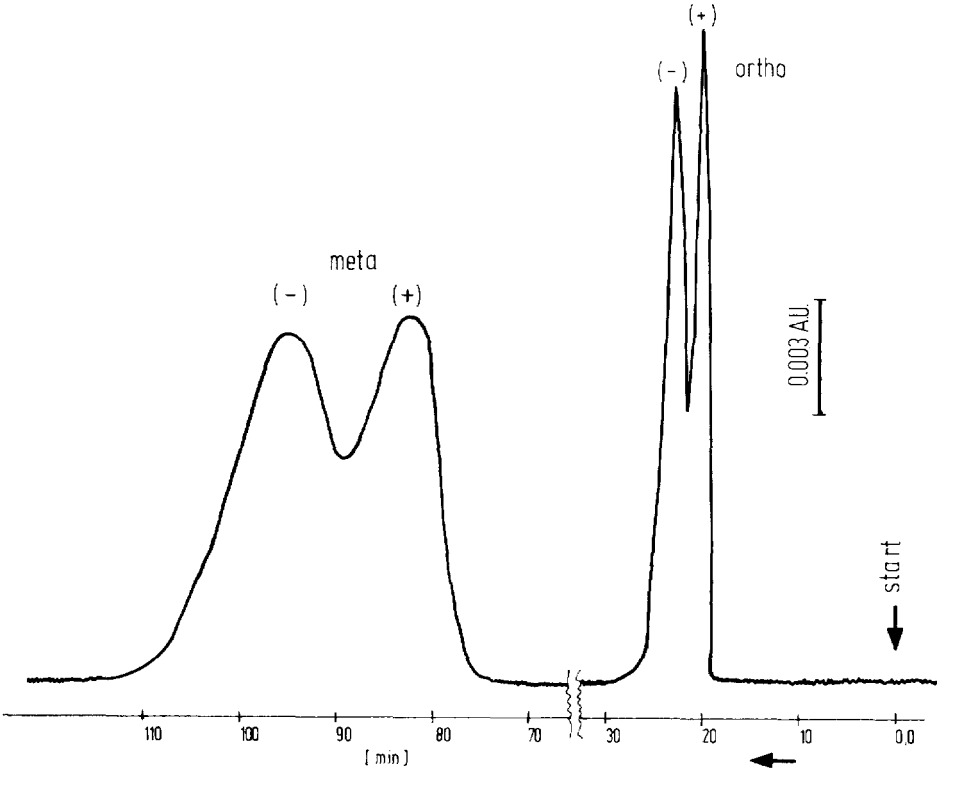

Fig. 2. Elution curve of a racemic mixture of *o*- and *m*-chloromandelic acids with an aqueous mobile phase of pH 6.8 and [*β*-CD] = $14.4 \cdot 10^{-3}$ *M*. Column: 250 × 4.5 mm I.D., LiChrosorb RP-18 (10 *μ*m); flow-rate, 1.2 ml/min.

separation of racemic *o*-chloromandelic acid presented in Fig. 1 was performed on a column only 5 cm long.

In the pH region corresponding to almost complete dissociation of the investigated acids (in this work pH 6.8), characterized by predominant participation of anions in the determination of the k' and α values, *o*- and *m*-chloromandelic acids in comparison with mandelic acid itself exhibited in the β-CD solutions a considerable increase in enantioselectivity (Fig. 2). This increase varied from $\alpha \approx 1.01$ for mandelic acid to $\alpha = 1.15$ for its *o*- and *m*-chloro derivatives; the enantioselectivity was nearly the same for both chloro-substituted mandelic acids.

According to the equation of Uekama *et al.*[9], and assuming that under the experimental conditions used here the active surface area of the reversed phase did not change with changes in β-CD concentration, we found that only 1:1 complexes were formed in the mobile phase solution.

The remarkable enantioselectivity observed for mandelic acid and for its *o*-chloro derivative at pH 2.1 in β-CD solutions, arising from differences in the stability constants [$K^0_{(+)}$ and $K^0_{(-)}$] and in the capacity factors [$k'_{(+)\mathrm{MH}\cdot\mathrm{CD}}$ and $k'_{(-)\mathrm{MH}\cdot\mathrm{CD}}$], was only observed for α-CD solution ($\alpha \approx 1.02$). A similar behaviour of α-CD, *i.e.*, a lack of enantioselectivity, was observed for *o*- and *m*-chloromandelic acids at pH 6.8. For mandelic acid and the derivatives investigated, β-CD seems to be a much more effective chiral host than α-CD.

Although these findings are of practical interest, they are very hard to interpret theoretically. Further studies on the inclusion of mandelic acid derivatives into β-CD, with other substituted guest molecules, are in progress.

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