Journal of Chromatography, 514 (1990) 19–27
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 544

Reversed-phase chromatographic separation of selected hydroxyl aromatics with β-cyclodextrin as a mobile phase additive

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(First received November 29th, 1989; revised manuscript received April 10th, 1990)

ABSTRACT

The capacity factors and selectivity factors of several pairs of hydroxyl aromatics were obtained with a C_{18} column with and without β-cyclodextrin in methanol–water mobile phases. In all but one case, the selectivity factors were improved. The hydroxyl aromatics chosen for the study were structurally similar and difficult to separate by reversed-phase chromatography. The relationship between α values and dissociation constants of the β-cyclodextrin-solute complexes was also considered and an approximate linear relationship was found between two parameters. In general, very substantial improvements were obtained for the separation of hydroxyl aromatics with β-cyclodextrin in the mobile phase.

INTRODUCTION

The separation of structural isomers and closely related compounds is very important in chromatography. The use of cyclodextrins in liquid chromatography has been shown to be effective in the separation of several compounds[1]. Cyclodextrins are oligosaccharides containing glucose units joined by α-1,4-linkages into a cone-shaped torus. α-Cyclodextrin (α-CD) is formed by the binding of six glucopyranose units, whereas β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) are formed by binding of seven and eight glucopyranose units, respectively. The glucopyranose units are chemically bonded such that the cavity that is formed is hydrophobic. The cavity is occupied by ether-like oxygen and C–H functionality. However, the outside of the cavity is hydrophilic due to hydroxyl groups. Molecules that have the correct sizes and shapes can fit inside of the cavity and form an inclusion complex and the inclusion complex can affect the retention of a solute in a chromatographic system. This is why cyclodextrins have been used in two different ways in high-performance liquid chromatography (HPLC), namely, either bonded to the stationary phase or dissolved in the mobile phase.

Dębowski and co-workers[1–4] reported the separation of mandelic acid into

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enantiomers, the separation of several isomers, and the separation of some aromatic amino acids by reversed-phase HPLC using α- or β-CD as a mobile phase component. Sybilska *et al.*[5] applied α- and β-CD to the separation of *o*-, *m*- and *p*-nitro-*cis*- and *trans*-cinnamic acids by reversed-phase HPLC. Gazdag *et al.*[6] reported the separation of optical isomers of D,L-norgestrel using α-, β- and γ-CD as mobile phase additives in HPLC. Gazdag *et al.*[7] applied α-, β- and γ-CD as mobile phase components in HPLC systems for the separation of isomeric estrogens in HPLC systems. Shimada *et al.*[8] discussed the use of cyclodextrins in the mobile phase for the separation of isomeric estrogens in HPLC systems. Sybilska *et al.*[9] discussed the use of α-CD for the separation of *o*-, *m*- and *p*-nitrobenzoic acids by reversed-phase HPLC. Dębowski *et al.*[10] studied the separation of isomeric alkylbenzenes in reversed-phase HPLC using aqueous mobile phases with α- and β-CD. In addition, β-CD and its derivatives have been used as selective agents for the separation of several compounds and isomers by HPLC[11–13]. Other aspects of β-CD in the mobile phase have also been considered[14].

In this work, the separation of several structurally similar compounds and structural isomers are reported that are normally very difficult to separate. Reversed-phase HPLC with methanol–water mobile phases and β-CD as a mobile phase additive, were used to separate the compounds.

EXPERIMENTAL

Apparatus

The liquid chromatograph used was a Waters unit with a Model 6000A pump (Waters Assoc., Milford, MA, U.S.A.), a U6K injector, a dual-channel ultraviolet detector set at 254 nm and a dual-channel 10-mV strip chart recorder. The column temperature was kept constant at 25°C by a Model FIAtron temperature controller. The column employed was a 10-μm Bondapak C_{18} (30 cm × 3.9 mm I.D.) purchased from Phenomenex.

Reagents

The model compounds were obtained from commercially available sources. The model compounds and β-CD were purchased from Aldrich (Milwaukee, WI, U.S.A.). Methanol was HPLC grade and obtained from Baker (Phillipsburg, NJ, U.S.A.).

Procedures

Methanol and water were prefiltered through a Millipore type FH 0.5 filter. β-Cyclodextrin was vacuum dried at 75–80°C for 8 h before use. Then, β-CD was dissolved in purified water and methanol was added to the β-CD solution. The model compounds were dissolved in methanol. The temperature was kept constant at 25°C and the column void volume was obtained by injecting a methanol solution of potassium nitrite.

RESULTS AND DISCUSSION

Separation of model compounds on a 10-μm C_{18} column

Previously, we investigated β-CD as a mobile-phase modifier in the reversed-

phase chromatography of polycyclic aromatic hydrocarbons, nitrogen heterocycles and hydroxyl aromatics[14]. A wide range of β-CD concentrations was employed and the retention properties of the compound classes were compared in methanol–water and ethanol–water mobile phases at 25°C. β-CD–solute dissociation constants were obtained for the compounds, and several conclusions were made about the mechanism of retention with β-CD in the mobile phase. In addition, comparisons were made between the structural features of the compounds and their capacity factors and dissociation constants. It was found that the retention properties of polycyclic aromatic hydrocarbons and nitrogen heterocycles were affected little by the presence of β-CD in the mobile because these solutes interacted more readily with the stationary phase rather than the β-CD in the mobile phase. Further, the retention characteristics of most of the hydroxyl aromatics were affected significantly with β-CD as a mobile phase modifier, indicating that β-CD could compete with the C_{18} column for the solute[14]. Because of our previous results with hydroxyl aromatics, it was decided to investigate the separation of structurally similar and isomeric hydroxyl aromatics with β-CD as a mobile phase modifier.

Fig. 1 shows the separation of 2,3-dihydroxynaphthalene and *p,p'*-biphenol in methanol–water (40:60) and in methanol–water (40:60) with 5.0 m*M* β-CD. In the absence of β-CD, the chromatographic bands of the two compounds overlapped severely and no separation was achieved. On the other hand, with β-CD in the mobile phase, the two compounds were easily separated and baseline resolution was obtained. This was due mainly to the formation of an inclusion complex between β-CD with *p,p'*-biphenol which resulted in a large decrease in the retention of *p,p'*-biphenol[14]. The separation of the model compounds can be related to their dissociation constants (K_D)

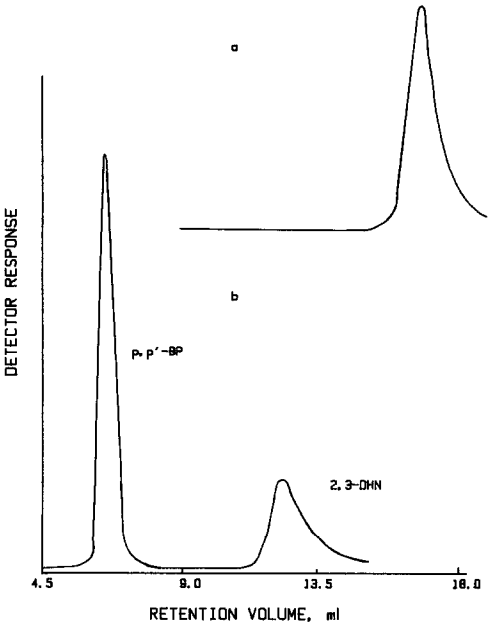

Fig. 1. Chromatograms of mixtures of 2,3-dihydroxynaphthalene (DHN) and *p,p'*-biphenol (*p,p'*-BP) in methanol–water (40:60) (a) and methanol–water (40:60) with 5.0 m*M* β-CD (b) on a 10-μm C_{18} column.

with β-CD. As discussed earlier, the smaller the K_D value, the smaller is the capacity factor (k')[14]. For instance, the K_D values of *p,p'*-biphenol and 2,3-dihydroxynaphthalene in methanol–water (40:60) with 5.0 m*M* β-CD are $4.10 \cdot 10^{-4}$ and $2.17 \cdot 10^{-3}$ *M*, respectively[14]. Thus, the decrease in k' for *p,p'*-biphenol would be larger than that for 2,3-dihydroxynaphthalene.

5-Indanol and *o,o'*-biphenol had very similar retention characteristics in methanol–water (40:60) so that the two peaks overlapped completely. With β-CD in the mobile phase, the separation of the mixture was easily achieved and baseline resolution was readily attained (Fig. 2). The K_D values for 5-indanol and *o,o'*-biphenol in methanol–water (40:60) are $1.41 \cdot 10^{-3}$ and $4.75 \cdot 10^{-3}$ M, respectively[14]. Since the K_D value of *o,o'*-biphenol is larger than that of 5-indanol, 5-indanol did not interact as readily with the stationary phase as did *o,o'*-biphenol. Other compound mixtures also showed good separations with β-CD present in methanol–water mobile phases. Some of these are shown in Figs. 3 and 4. Other compound pairs that showed excellent separation with β-CD in the mobile phase were 1,7-dihydroxynaphthalene and *p,p'*-biphenol, and 2-naphthol and *o,o'*-biphenol.

Separation of structural isomers

The separation of structural isomers in HPLC is of great importance. In this work, a mixture of 2-, 3- and 4-phenylphenol showed only two peaks in methanol–water (40:60). One peak belonged to 2-phenylphenol, and the other peak consisted of 3- and 4-phenylphenol. However, three peaks with baseline resolutions were obtained in methanol–water (40:60) containing 5 m*M* β-CD. The chromatograms are shown in Fig. 5a and b. The chromatograms indicated that the retention of 4-phenylphenol was

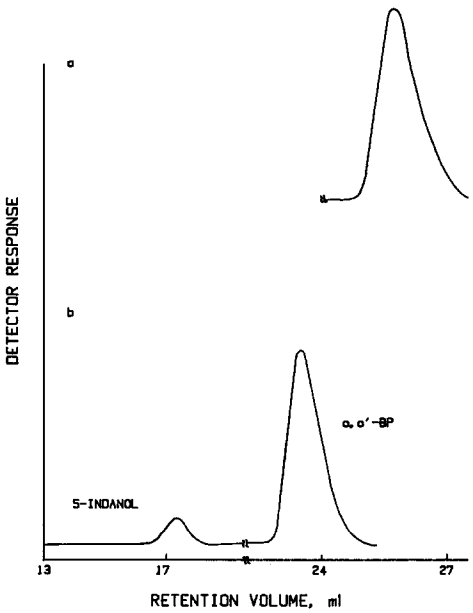

Fig. 2. Chromatograms of mixtures of 5-indanol and *o,o'*-biphenol (*o,o'*-BP) in methanol–water (40:60) (a) and methanol–water (40:60) with 5.0 m*M* β-CD (b) on a 10-μm C_{18} column.

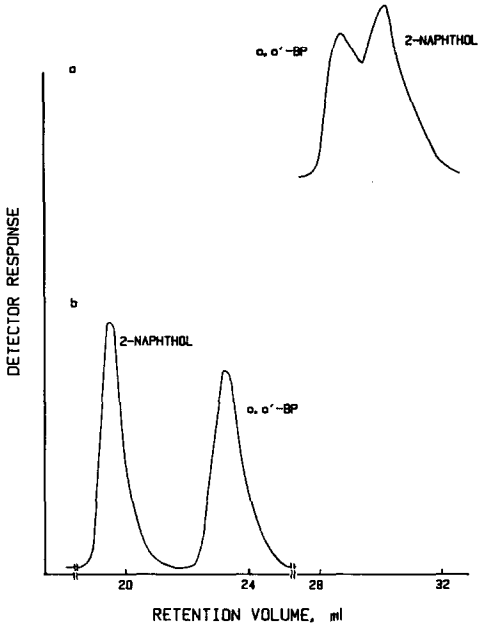

Fig. 3. Separation of *o,o'*-biphenol (*o,o'*-BP) and 2-naphthol in methanol–water (40:60) without (a) and with (b) 5.0 m*M* *β*-CD on a 10-*μ*m C_{18} column.

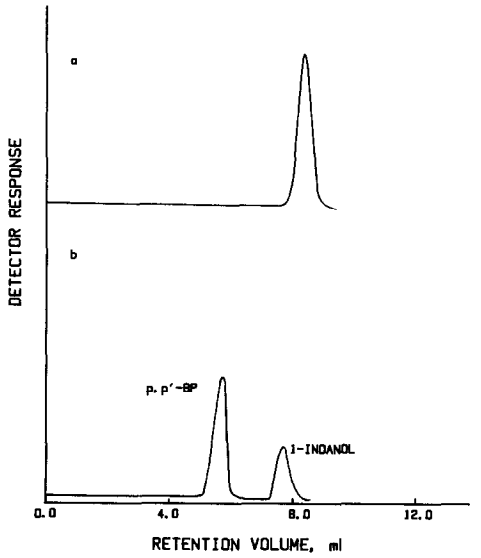

Fig. 4. Chromatograms of mixtures of *p,p'*-biphenol and 1-indanol in methanol–water (50:50) (a) and methanol–water (50:50) with 4.0 m*M* *β*-CD (b) on a 10-*μ*m C_{18} column.

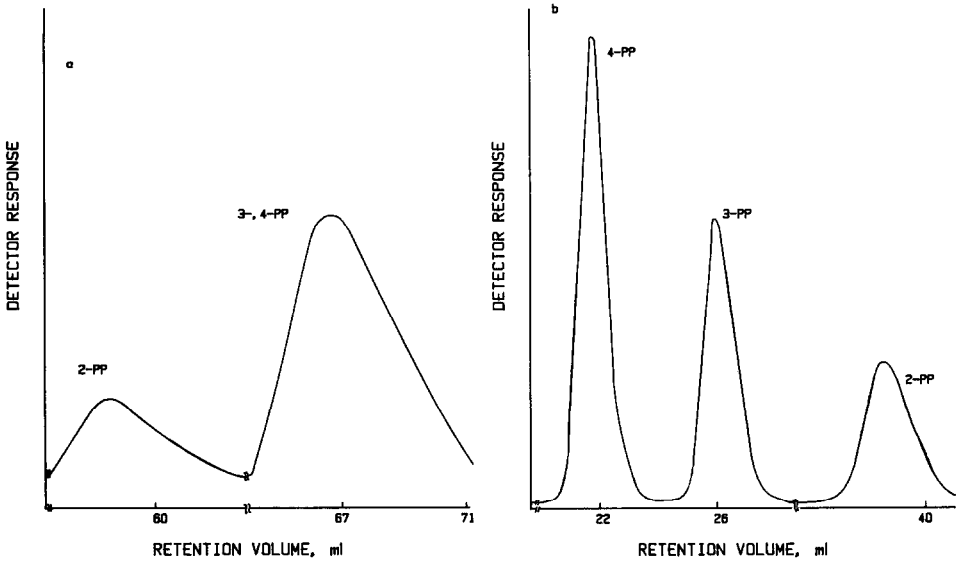

Fig. 5. Separation of phenylphenols in methanol–water (40:60) without (a) and with 5.0 m*M* β-CD (b) on a 10-μm C_{18} column.

substantially reduced and was the least retained of the isomers with β-CD in the mobile phase (Fig. 5). The separation resulted because a stronger β-CD inclusion complex formed with 4-phenylphenol than with 2- and 3-phenylphenol. The changes in k' values for 2-, 3- and 4-phenylphenol, due to the addition of β-CD, were related to their K_D values which are $1.74 \cdot 10^{-3}$, $6.18 \cdot 10^{-4}$ and $4.72 \cdot 10^{-4}$ M, respectively[14]. It can be seen that 4-phenylphenol has the smallest K_D value, and therefore it would be expected to have smaller k' values with β-CD in the mobile phase compared to the other phenylphenol isomers.

Selectivity factors

The selectivity factor (α) for the model compounds in methanol–water mixtures with β-CD can be obtained from the following equation,

$$\alpha = \frac{k'_{02}K_{D2}}{k'_{01}K_{D1}}\left(1 + \frac{K_{D1} - K_{D2}}{K_{D1} + [CD]_m}\right)$$

where k'_{01} and k'_{02} are the capacity factors of components 1 and 2 in the absence of β-CD, K_{D1} *and* K_{D2} are the dissociation constants of the inclusion complexes of components 1 and 2 with β-CD, respectively, and $[CD]_m$ is the equilibrium concentration of β-CD. The above equation is similar to the equation used by Fujimura *et al.*[15]. However, they used total β-CD concentration in the equation. We have discussed the use of the equilibrium concentration in relating k' values to K_D values with β-CD in the mobile phase[14]. Table I lists the capacity factors and selectivity factors of several pairs of compounds in different methanol–water mobile phases with β-CD on a 10-μm column. As shown in Table I, the α values increased with addition of

TABLE I

CAPACITY FACTORS AND SELECTIVITY FACTORS FOR PAIRS OF HYDROXYL AROMATICS WITH β-CYCLODEXTRIN PRESENT IN THE MOBILE PHASE

Compound pairs[a]	*Methanol–water*	*k'*	α[b]
2,3-DHN	40:60	3.01	1.67
p,p'-BP	40:60	1.80	
1-Indanol	40:60	3.88	1.00
1,7-DHN	40:60	3.88	
5-Indanol	40:60	6.25	1.43
o,o'-BP	40:60	8.92	
1-Indanol	50:50	7.75	1.67
p,p'-BP	50:50	5.60	
o,o'-BP	30:70	15.82	1.50
2-naphthol	30:70	10.52	
3-PP	40:60	6.22	1.22
4-PP	40:60	5.10	
p,p'-BP	57:43	0.958	1.21
1-DHN	57:43	1.16	
1-Indanol	60:40	1.31	1.31
1,7-DHN	60:40	0.997	

[a] DHN = Dihydroxynaphthalene; BP = biphenol; PP = phenylphenol.

[b] Without β-CD in the mobile phase the α values were equal to 1.00, except for *o,o'*-BP and 2-naphthol, and 1-indanol and 1,7-DHN.

β-CD, except for one pair of compounds. As an example, no separation was achieved for the mixture of *p,p'*-biphenol and 2,3-dihydroxynaphthalene ($\alpha = 1.00$), whereas baseline resolution was attained with the addition of β-CD ($\alpha = 1.67$). As another example, the α value improved from 1.00 with no β-CD to 1.43 with 5.0 m*M* β-CD in methanol–water (40:60) for the separation of 5-indanol and *o,o'*-biphenol. The α values listed in Table I were the same for a given pair of compounds whether they were calculated with the *k'* values for the pure compounds or calculated from *k'* values obtained from a mixture of a given pair of compounds.

It is instructive to make a comparison between the change in the selectivity factors of pairs of compounds with and without β-CD and the corresponding ratio of their K_D values. Fig. 6 shows the relationship between $\Delta\alpha$ and the ratio of K_D values in methanol–water (40:60) for four pairs of compounds. The $\Delta\alpha$ value refers to the difference in the selectivity factor of a pair of components in a mixture with and without β-CD in the mobile phase. The points in Fig. 6 represent mixtures of 3- and 4-phenylphenol (point 1), 2-naphthol and *o,o'*-biphenol (point 2), 5-indanol and *o,o'*-biphenol (point 3) and *p,p'*-biphenol and 2,3-dihydroxynaphthalene (point 4). Points 1, 3 and 4 fell on a straight line. As shown in Fig. 6, point 2 did not fall near the line. The linear relationship indicated that as the K_D ratio increased, the difference in selectivity factors increased and better separation was achieved. *p,p'*-Biphenol and 2,3-dihydroxynaphthalene (point 4) were separated with a larger $\Delta\alpha$ value compared to

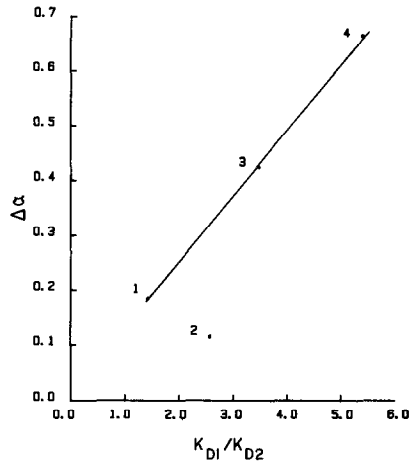

Fig. 6. Plot of $\Delta\alpha$ *vs.* the K_D ratio for several pairs of hydroxyl aromatics in methanol–water (40:60) on a 10-μm C_{18} column. See text for discussion of data points.

5-indanol and *o,o'*-biphenol and 3- and 4-phenylphenol in methanol–water (40:60). These results indicated that the separation of the model compounds was related to their ability to form inclusion complexes with the β-CD in methanol–water (40:60) and followed a similar chromatographic mechanism. However, other types of interactions were occurring for either 2-naphthol and *o,o'*-biphenol as indicated by the fact that point 2 did not fall on the line in Fig. 6.

CONCLUSIONS

The addition of β-CD to the mobile phase in the reversed-phase chromatographic system investigated resulted in dramatic improvements in the separation of structurally similar hydroxyl aromatics. Without β-CD in the mobile phase, many of the pairs of compounds could not be separated with methanol–water or were only partially separated. However, with β-CD the compound pairs were easily separated. With the phenylphenol isomers, only two chromatographic bands were obtained with methanol–water. However, by the addition of β-CD to the mobile phase, the phenylphenol isomers were readily separated and baseline resolution was obtained for the isomers. The primary conclusion from this work is that with β-CD in the mobile phase very significant improvements in the separation of hydroxyl aromatics can be achieved.

ACKNOWLEDGEMENTS

This research was partially supported by Burdick and Jackson Laboratories and by the U.S. Department of Energy under contract No. DE-AC22-83PC60015 and under Grant No. DE-FG-02-86ER13547.

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