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COMPARATIVE STUDIES OF THE ABILITY OF CYCLOMALTO-HEXAOSE, -HEPTAOSE, AND -OCTAOSE TO FORM INCLUSION COMPLEXES WITH ISOMERS OF SOME HYDROCARBONS UNDER THE CONDITIONS OF GAS–LIQUID CHROMATOGRAPHY

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ABSTRACT

Solutions of cyclomalto-hexaose (αCD), -heptaose (βCD), and -octaose (γCD) in formamide were used as stationary phases in g.l.c. βCD was highly selective towards *para*- and *meta*-dialkylbenzenes, *cis*- and *trans*-1,2-dimethylcyclohexanes, and *cis*- and *trans*-decalins. γCD exhibited a substantial affinity for the *ortho*-isomers of dialkylbenzenes and 1,2,3-trimethylbenzene, and was the most effective in differentiating between isomeric butylbenzenes. Only αCD recognized the enantiomers of α- and β-pinene, and optically active *cis*- and *trans*-pinane. There was no relationship between the strength of the complexation by αCD, βCD, and γCD and the ability of selective differentiation of isomeric compounds.

INTRODUCTION

Cyclomalto-oligosaccharides (cyclodextrins, CDs) have found application in liquid and gas chromatography[1–4], isotachophoresis[5,6], and electrokinetic chromatography[7]. In g.l.c., CDs and their *O*-alkylated or *O*-acylated derivatives have been used as modifiers of both solid[8–11] and liquid[12–22] stationary phases. Polymeric stationary phases incorporating CDs have also been used[23].

Chromatographic data can furnish new evidence concerning the inclusion properties of CDs, as well as the stoichiometry and stability of the complexes.

The aim of the present studies was to compare the complexation of hydrocarbons by solutions of cyclomalto-hexaose (αCD), -heptaose (βCD), and -octaose (γCD) in formamide under the conditions of g.l.c. G.l.c. is characterized by high reversibility and a large range of linear isotherms that allows recognition of the regio- and stereo-selectivity imparted to the liquid stationary phase by complexation with CDs. However, the use of this approach requires the influence of the

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solvent–matrix medium to be considered, and structural, geometrical, and optical isomers of representative hydrocarbons have been studied.

EXPERIMENTAL

Reagents. — CDs were supplied by Chinoin (Budapest), and Celite (30–80 mesh) for g.l.c. was from B.D.H. (Poole). All compounds investigated were commercial products and were used without further purification.

Apparatus and procedures. — A Hewlett–Packard 5890 gas chromatograph was used together with a flame-ionization detector and glass columns (2 m × 4 mm i.d.). The compounds (0.02 μL) were injected with Hamilton microsyringes either separately or as mixtures.

The stationary phases were prepared as follows. An aqueous solution of the CD and formamide was deposited on Celite. The resulting slurry was shaken for ~10 min and the excess of water was then slowly evaporated at 40°/20 mmHg. The quantities of formamide (4.54 g) and Celite (20 g) were constant. Columns contained CDs as follows: I, zero (control); II, αCD (0.6 g); III, βCD (1.2 g); IV, βCD (0.32 g); V, γCD (1.2 g), VI, γCD (0.33 g).

In contrast to βCD and γCD, αCD is an efficient separating agent under the conditions of g.l.c. when the formamide solution contains 3–4% of water. Consequently, the stationary phase for column II contained 4% of water, and $LiNO_3$ (0.45 g) was added as the stabilizing agent. The contents of formamide and water in the final stationary phase were assayed by thermogravimetric analyses with a Du Pont apparatus. The amount of coated support in each column was 11.5 ±0.5 g.

Stabilities of CD complex were compared using the equation

$$t'_{R(CD)} = t'_{R(F)} (1 + K[CD]),$$

where $t'_{R(CD)}$ and $t'_{R(F)}$ are the adjusted retention times* of the solute on the column containing the CD in formamide and of the solute on the reference column containing pure formamide, respectively, K is the stability constant of the 1:1 guest–CD complex, and [CD] is the concentration of CD in solution in the formamide. This equation is true only if 1:1 guest–CD complexes are formed.

The separation factor α for two solutes A and B was evaluated as the ratio of their adjusted retention times ($\alpha = t'_{R(B)}/t'_{R(A)}$) when the same column and identical conditions of separation were used.

A constant inlet pressure (2.75 ±0.05 atm.) and helium flow-rate (50 ±0.5 mL/min) were maintained. Under these conditions, it was possible to compare the stability constants of various CD complexes, although their exact values could not be determined[24,25].

*Given by the equation $t'_R = t_R - t_0$, where t_R denotes the observed retention time and t_0 is the dead time of the column, corresponding to elution of the volume of its mobile phase.

RESULTS AND DISCUSSION

Butylbenzenes. — The results with *n*-, iso-, *sec*-, and *tert*-butylbenzenes are shown in Table I.

βCD had an exceptionally high affinity for *tert*-butylbenzene, which was eluted after *n*-butylbenzene on column III (βCD) and before *n*-butylbenzene on column I (control). However, column V (γCD) afforded the best separation of the four isomers, and stabilities of the complexes with βCD were much higher than those with γCD.

Dialkylbenzenes. — The results for the *ortho, meta,* and *para* isomers of ethyl-methyl, methyl-isopropyl, and diethyl benzene are recorded in Table II.

Only βCD distinctly differentiated *m*- and *p*-dialkylbenzenes (Fig. 1). This behaviour could find analytical application because these isomers are practically inseparable by conventional partition chromatography (*cf.* the results obtained using columns I and III).

On the other hand, γCD exhibited a substantial affinity for *o*-dialkylbenzenes

TABLE I

ADJUSTED RETENTION TIMES (t'_R IN MIN) OF THE STRUCTURAL ISOMERS OF BUTYLBENZENE, DETERMINED BY G.L.C. AT 60°

Compound	*Column I*	*Column II (αCD)*	*Column III (βCD)*	*Column V (γCD)*
Bu^n-Ph	5.1	13.0	40	11.5
Bu^i-Ph	3.5	7.6	47	8.8
Bu^s-Ph	3.8	8.5	43	13.8
Bu^t-Ph	4.1	8.0	101	16.5

TABLE II

ADJUSTED RETENTION TIMES (t'_R IN MIN) OF THE STRUCTURAL ISOMERS OF SOME DIALKYLBENZENES DETERMINED BY G.L.C. AT 60°

Compound	*Column I*	*Column II (αCD)*	*Column III (βCD)*	*Column V (γCD)*
o-MeC_6H_4Et	5.2	6.1	16.5	21.0
m-MeC_6H_4Et	3.6	4.9	10.3	7.6
p-MeC_6H_4Et	3.6	5.0	13.9	6.6
o-$MeC_6H_4Pr^i$	6.0	6.5	27.0	26.0
m-$MeC_6H_4Pr^i$	4.2	4.3	24.5	9.0
p-$MeC_6H_4Pr^i$	4.2	4.2	36.0	8.3
o-$Et_2C_6H_4$	6.7	5.2	24.0	28.0
m-$Et_2C_6H_4$	5.0	4.1	15.0	10.5
p-$Et_2C_6H_4$	5.0	4.1	27.0	11.0

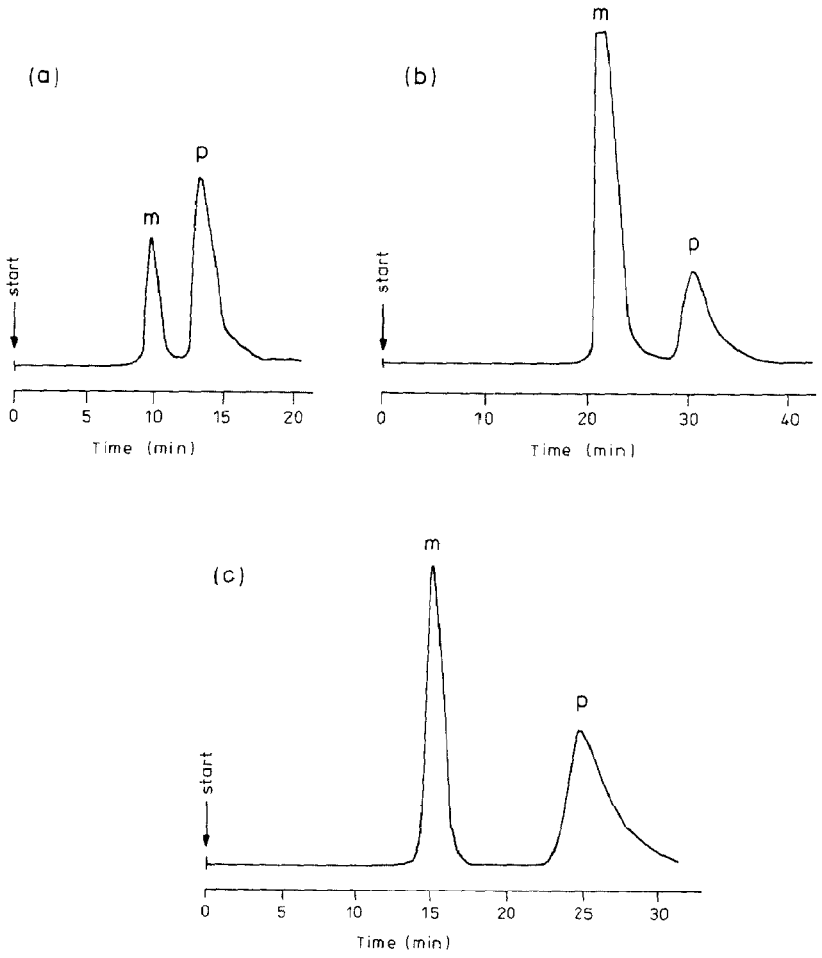


Fig. 1. Chromatograms of mixtures of *m*- and *p*-isomers of (a) ethylmethylbenzene, (b) methylisopropylbenzene, and (c) diethylbenzene, obtained at 60° on column III (βCD).

TABLE III

ADJUSTED RETENTION TIMES (t'_R IN MIN) OF ISOMERIC TRIMETHYLBENZENES DETERMINED BY G.L.C. AT 60°

Isomer	*Column I*	*Column II (αCD)*	*Column III (βCD)*	*Column V (γCD)*
1,3,5	3.8	3.0	5.1	6.5
1,2,4	5.4	4.2	15.0	11.3
1,2,3	8.4	6.5	26.0	44.0

TABLE IV

ADJUSTED RETENTION TIMES (t'_R IN MIN) OF *cis* AND *trans* ISOMERS DETERMINED BY G.L.C. AT 40° AND/OR 60°

Compound	*Column I*		*Column II (αCD)*		*Column III (βCD)*		*Column IV (βCD)*		*Column V (γCD)*		*Column VI (γCD)*
	40°	*60°*	*40°*	*60°*	*40°*	*60°*	*40°*	*60°*	*40°*	*60°*	*60°*
1,2-Dimethyl-cyclohexane											
cis	0.6		2.1		18.0		5.3		10.5		
trans	0.6		0.7		5.2		1.6		8.4		
Decalin											
cis		2.2		3.1				71		>100	19
trans		1.4		2.2				18		41	9
Pinane											
(−)-*cis* (**1**)	1.9	0.7	10.3			53				26	
(+)-*trans* (**2**)	1.7	0.6	2.6			57				15	

as reflected by the adjusted retention times which were much higher than those of the *m*- and *p*-isomers.

Trimethylbenzenes. — The results for 1,2,3-, 1,2,4-, and 1,3,5-trimethylbenzene are recorded in Table III.

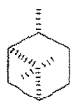
As with the *o*-dialkylbenzenes, γCD had a much higher affinity for 1,2,3-trimethylbenzene than for the 1,2,4- and 1,3,5-isomers. On the other hand, although the complexes with βCD were less stable, the high selectivities of complexation make βCD the most promising separating agent in g.l.c.

Geometrical isomers. — The results for the *cis* and *trans* forms of 1,2-dimethylcyclohexane, decalin, and pinane (**1** and **2**, respectively) are recorded in Table IV.

βCD had the highest selectivity for 1,2-dimethylcyclohexane and mediated a good separation of the isomers. Similarly, mixtures of *cis*- and *trans*-decalin could be resolved using βCD, but only in the presence of a low concentration of the separating agent (column IV). γCD exhibited an extremely high affinity for *cis*-decalin, but the *cis–trans* selectivity was lower than with βCD. The (−)-*cis* (**1**) and (+)-*trans* (**2**) isomers of pinane were separated efficiently using columns modified with αCD. Although the αCD complexes had relatively low stability, the selectivity was much higher than with βCD and γCD.

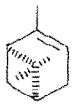
Enantiomers of α- and β-pinene. — The results with these compounds are recorded in Table V.

Only αCD distinctly recognized the enantiomers (**3** and **4**) of α-pinene. The enantioselectivity for β-pinene (**5** and **6**) was slightly lower, but sufficient for resolution under similar conditions. There was no enantioselectivity for βCD and γCD, albeit their complexes with α- and β-pinene were much more stable than those with αCD.



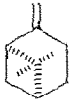
1(−)-*cis*-pinone

2(+)-*trans*-pinone



3(−)-α-pinene

4(+)-α-pinene



5(−)-β-pinene

6(+)-β-pinene

Table VI shows some selected separation factors (α) of the isomeric compounds investigated.

A comparison of the results in Tables I–V with those presented in Table VI reflects the widely different abilities of αCD, βCD, and γCD to include various isomeric hydrocarbons, although the results do not allow generalizations to be made. Also, there is no relationship between the strength of complexation of various organic compounds by αCD, βCD, and γCD and their ability to differentiate selectively the isomers of these compounds.

TABLE V

ADJUSTED RETENTION TIMES (t_R' IN MIN) OF THE ENANTIOMERS OF α- AND β-PINENE DETERMINED BY G.L.C. AT 40° AND/OR 60°

Compound	*Column I*		*Column II (αCD)*	*Column III (βCD)*	*Column V (γCD)*	
	40°	*60°*	*40°*	*60°*	*40°*	*60°*
(−)-α-Pinene (**3**)	1.3	0.6	9.4	25	36	13
(+)-α-Pinene (**4**)	1.3	0.6	4.2	25	36	13
(−)-β-Pinene (**5**)	3.0	1.3	8.2	62	92	31
(+)-β-Pinene (**6**)	3.0	1.3	5.5	62	92	31

TABLE VI

SOME SELECTED SEPARATION FACTORS (α) OF THE ISOMERS INVESTIGATED, DETERMINED BY G.L.C. AT 40° AND/OR 60°

Isomers	*Column I*		*Column II* (αCD)		*Column III* (βCD)		*Column IV* (βCD)		*Column V* (γCD)		*Column VI* (γCD)
	40°	*60°*	*40°*	*60°*	*40°*	*60°*	*40°*	*60°*	*40°*	*60°*	*60°*
Bu^n/Bu^t Benzene		1.24		1.63		0.4				0.7	
p/m MeC_6H_4Et		1.0		1.02		1.35				0.87	
p/m $MeC_6H_4Pr^i$		1.0		0.98		1.47				0.92	
p/m $Et_2C_6H_4$		1.0		1.0		1.8				1.05	
o/p MeC_6H_4Et		1.44		1.22		1.19				3.33	
o/p $MeC_6H_4Pr^i$		1.43		1.55		0.75				3.13	
o/p $Et_2C_6H_4$		1.34		1.27		0.89				2.55	
1,2,3/1,2,4 $Me_3C_6H_3$		1.56		1.55		1.73				3.89	
1,2,4/1,3,5 $Me_3C_6H_3$		1.42		1.40		2.94				1.74	
cis/trans 1,2-Dimethyl-cyclohexane	1.0		3.0		3.46		3.31		1.25		
cis/trans Decalin		1.57		1.41				3.94		>2.4	
(−)-*cis*/(+)-*trans* Pinane	1.12	1.17	3.96			0.9				1.7	2.1
(−)/(+) α-Pinene	1.0	1.0	2.24			1.0			1.0	1.0	
(−)/(+) β-Pinene	1.0	1.0	1.49			1.0			1.0	1.0	

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