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PREPARATION AND RETENTION BEHAVIOUR OF CHEMICALLY BONDED METHYLATED-CYCLODEXTRIN STATIONARY PHASES FOR LIQUID CHROMATOGRAPHY

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SUMMARY

Both *a*- and β-cyclodextrin were converted into the ethylenediamine monosubstituted per-O-methyl derivatives, and the latter were coupled to succinamidopropyl silica. The resulting methylated a-cyclodextrin stationary phase improves the separation of the *ortho*, *meta* and *para* isomers of several disubstituted benzene derivatives, compared with the unmodified a-cyclodextrin stationary phase. Methylation of β-cyclodextrin, however, results in less good selectivity in the separation of the three isomers. The methylated β-cyclodextrin stationary phase can efficiently separate the antiepileptic drugs or the substituted naphthalene derivatives.

INTRODUCTION

Recently, chemical modification of cyclodextrins with various functional groups has been extensively investigated in an attempt to improve the complexing and catalytic abilities of cyclodextrins. Various functional groups have been introduced onto their rim', bringing about changes in the depth of the cyclodextrin cavity, in the hydrogen-bonding ability and in various other physical properties. X-Ray structural investigation has shown that the host-guest interaction in complexes of per-0-methylated a-cyclodextrin is different from that in those of unmodified α-cyclodextrin, in which the guest molecule of benzaldehyde or p-nitrophenol is positioned the other way round. This change is ascribed to the difference in the shape and size of the host cavity[2].

In previous papers[3–5], we reported the specific, liquid chromatographic separations of some aromatic compounds on unmodified and acylated cyclodextrin stationary phases; acylation resulted in selectivity changes in solute retention, as expected. Per-0-methylated cyclodextrins readily dissolve not only in water but also in organic solvents, in addition to the above-mentioned change in the inclusion. More-

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over, it has been reported that the complexes of methylated cyclodextrins are usually more stable than the corresponding complexes of unmodified cyclodextrins[6]. Therefore, the retention behaviour of methylated cyclodextrin stationary phases is of great interest, because changes in solute retention are expected.

In this work, ethylenediamine monosubstituted per-O-methyl-a- and -β-cyclodextrins were prepared for the first time. Each of these novel cyclodextrin derivatives was immobilized on silica particles. The retention behaviour of some aromatic compounds on these stationary phases was studied and compared with that on the unmodified cyclodextrin stationary phases.

EXPERIMENTAL

Materials and chromatography

Silica gel (Wakogel LC-10H, 10-μm particle size) and all other reagents were purchased from Wako (Osaka, Japan).

All chromatographic studies were carried out with' a system consisting of a Model KHD-W-52 pump, a Type KD-I pulse damper, a Model KHP-UI-130A injector (Kyowa Seimitsu, Tokyo, Japan) and a Model UVILOG-7 variable-wavelength UV detector (Oyo-Bunko, Tokyo, Japan).

Each stationary phase was packed into a stainless-steel column (15 × 0.4 cm I.D.). The flow-rate of eluent (water, 1/15 *M* phosphate buffer, methanol-water or methanol-l/l 5 *M* phosphate buffer) was 1 .O ml/min. The concentration of the sample solutes was 0.2 m*M* except for benzene (1 m*M*), and a volume of 20 μl was injected.

The detection wavelength was 254 nm (disubstituted benzenes and antiepileptic drugs) or 205 nm (other solutes).

Preparation of methylated cyclodextrin stationary phases

Ethylenediamine monosubstituted per-0-methyl-a-cyclodextrin (Me-a-en) and -β-cyclodextrin (Me-/I-en) (i.e. mono-[6-deoxy-6-N-(2-aminoethyl)amino]-per-O-methyl-a-cyclodextrin and -β-cyclodextrin, respectively) were prepared according to the scheme shown in Fig. 1. Mono-(6-O-trityl)-a- or -β-cyclodextrin (2) was obtained' by reaction of *a*- or β-cyclodextrin (1) with trityl chloride (Tr-Cl) in pyridine by a modification of the method of Melton and Slessor[7]. All the remaining hydroxyl groups were fully methylated using methyl iodide and sodium hydride in dimethylformamide[8]. Removal of the trityl group, by brief treatment of (3) in a concentrated hydrochloric acid-chloroform system, gave (4). Reaction of the free hydroxyl group withp-toluenesulphonyl chloride (Ts-Cl) in pyridine, followed by displacement of the sulphonate group with ethylenediamine, gave Me-a-en or Me-β-en (6).

^{1}H-NMR δ (in C^2HCl_3): 2.75 (6H, NCH_2); 3.45 (15H, O_6-CH_3); 3.50 (18H, O_3-CH_3); 3.65 (18H, O_2-CH_3) for Me-a-en: 2.73 (6H, NCH_2); 3.38 (18H, O_6-CH_3); 3.48 (21H, O_3-CH_3); 3.62 (21H, O_2-CH_3) for Me-P-en.

Analysis: calculated for $C_{55}H_{100}O_{29}N_2$ (Me-a-en): C, 52.70; H, 8.04; N, 2.24; found: C, 52.93; H, 8.07; N, 2.12; calculated for $C_{64}H_{116}O_{34}N_2$ (Me-p-en): C, 52.74; H, 8.02; N, 1.92; found: C, 52.19; H, 8.02; N, 1.91.

Me-a-en or Me-P-en was coupled to succinamidopropyl silica (Su-Silica) as follows. In 40 ml of 0.15 *M* phosphate buffer (pH 5.5), 1 g of (6) was dissolved, and the pH of the solution was adjusted to 5.5 with hydrochloric acid. Su-Silica (2 g) was

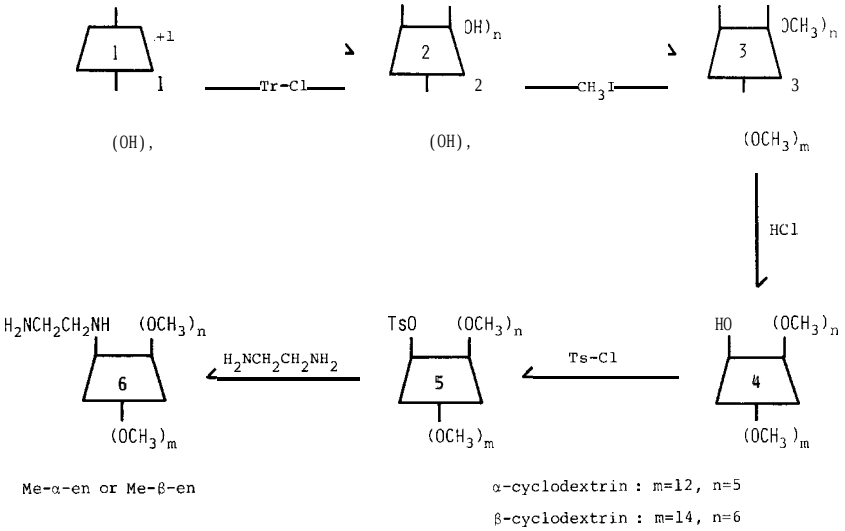

Fig. 1. Preparation of ethylenediamine monosubstituted per-0-methyl-cyclodextrins.

suspended in this solution, and 0.5, 0.3 and 0.2 g of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride were added at intervals of 2 h with stirring. After the mixture had been stirred for 12 h, the bonded phase was filtered off, washed thoroughly with water and then several times with methanol and diethyl ether, and finally dried *in vacuo* at 80°C for 24 h.

The unmodified cyclodextrin stationary phases were also prepared in much the same way, using ethylenediamine monosubstituted cyclodextrins instead of (6). The amounts of cyclodextrin immobilized were evaluated by elemental analysis and are given in Table I, together with the codes of the cyclodextrin stationary phases prepared.

RESULTS AND DISCUSSION

We used several methods in an attempt to methylate unmodified cyclodextrins immobilized in advance on silica gel, but in vain. We obtained the methylated cyclodextrin stationary phases by coupling the ethylenediamine monosubstituted per-0-methyl-cyclodextrin derivatives to succinamidopropyl silica as described in the Experimental section.

TABLE I

CYCLODEXTRIN CONTENTS IN THE STATIONARY PHASES

Phase	*Cyclodextrin (CD)*	*Cyclodexlrin content (μmol/g)*
a-en-%-Silica	a-CD	88.0
Me-a-en-k-Silica	Methylated a-CD	78.7
β-en-Su-Silica-(1)	β-CD	72.7
β-en-Su-Silica-(2)	β-CD	39.2
Me-β-en-Su-Silica	Methylated /?-CD	37.4

Dependence of retention upon eluent

The retention of six disubstituted benzene isomers (cresol, iodoaniline, toluidine, nitroaniline, nitrophenol or dinitrobenzene) decreases on the methylated cyclodextrin stationary phases, as the methanol content in the eluent of methanol-water increases. Fig. 2 shows a typical example, the o-, *m*- and p-isomers of cresol both on the methylated and unmodified cyclodextrin stationary phases. It is apparent from the amounts of cyclodextrin (methylated < unmodified, see Table I) and methanol in the eluent that the methylated cyclodextrin stationary phases retain the cresol isomers more strongly than do the unmodified ones. In particular, Me-a-en-Su-Silica interacts strongly. This trend is true for the other solutes. The three isomers of iodoaniline, nitroaniline or nitrophenol are not eluted from Me-a-en-Su-Silica with methanol-water (20:80) within 60 min.

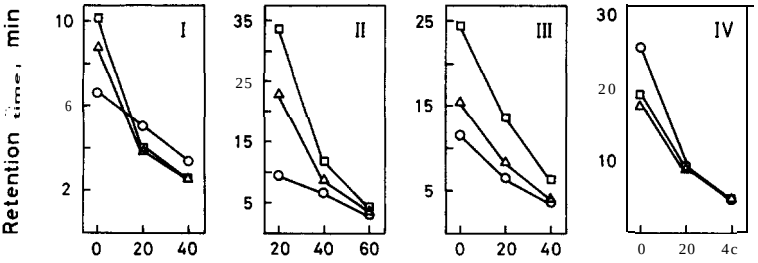

Fig. 2. Effect of methanol content in the eluent on retention times of cresol isomers (0 = *ortho*; A = *meta* and ❑ = *para*) on cyclodextrin stationary phases (I = a-en-Su-Silica; II = Me-a-en-Su-Silica; III = β-en-Su-Silica-(1); IV = Me-β-en-Su-Silica).

Methanol-phosphate buffer at pH 6.7, 5.6 or 4.5 was used as eluent in order to investigate the effect of pH on the retention. The retention of cresol, iodoaniline, nitroaniline and dinitrobenzene was only slightly affected, whereas that of nitrophenol, aminobenzoic and nitrobenzoic acids and toluidine was strongly affected. Fig. 3 shows the results for nitrophenol, aminobenzoic acid and toluidine, both on a-en-Su-Silica and Me-a-en-Su-Silica. The retention of aminobenzoic and nitrobenzoic acids decreases, whereas that of toluidine increases, with increasing pH on all the cyclodextrin stationary phases studied. This phenomenon can be reasonably explained by the well-known findings that the inclusion of charged groups (such as O-, CO;, SO; and NH_3^+) into the cyclodextrin cavities is not favoured; exceptionally, nitrophenolate interacts with the cyclodextrin cavities more strongly than the neutral form of nitrophenol does[9]. In fact, the retention of nitrophenol increases with increasing pH on the cyclodextrin stationary phases, except for Me-a-en-Su-Silica (Fig. 3). This abnormal behaviour of Me-a-en-Su-Silica may be due to a different orientation of nitrophenol in the methylated a-cyclodextrin cavity from that in the other cyclodextrin cavities. Further work is needed to explain this selectivity change in the retention.

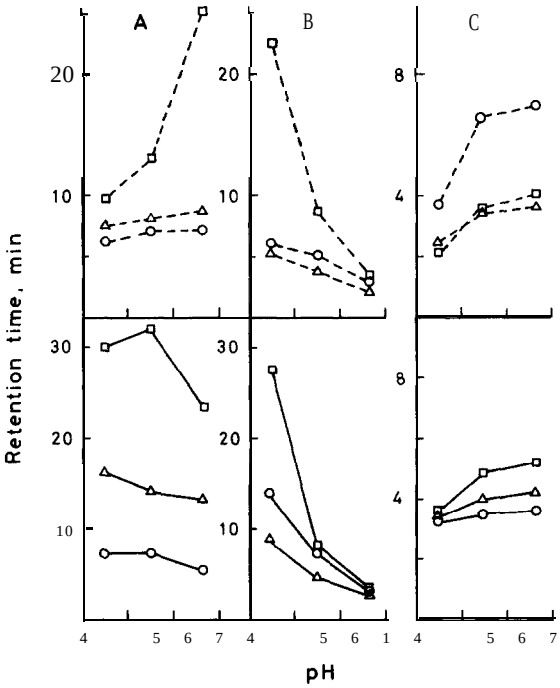

Fig. 3. Effect of pH of the eluents on retention times of nitrophenol (A), aminobenzoic acid (B) and toluidine (C) isomers (0 = *ortho*; A = *meta* and □ = *para*). Stationary phases: —— = Me-α-en-Su-Silica in methanol–1/15 *M* phosphate buffer (40:60), except for nitrophenol (methanol-l/75 *M* phosphate buffer (50:50)); ----- = a-en-Su-Silica in methanol-l/15 *M* phosphate buffer (10:90).

Retention of disubstituted benzene derivatives

Fig. 4 shows the retention times of six disubstituted benzene derivatives, both on the methylated and unmodified cyclodextrin stationary phases. The separations of the three isomers of cresol, iodoaniline, toluidine and dinitrobenzene are improved by the methylation of a-cyclodextrin. On the other hand, apparently, the methylation of β-cyclodextrin results in less good selectivity. We assume that these selectivity changes can be ascribed to changes in the size of the cyclodextrin cavities: methylation enlarges the secondary side of the cyclodextrin cavity and distorts the cavity[2,10]. The disubstituted benzene derivatives can fit the methylated a-cyclodextrin cavity, whereas they are too small to be in intimate contact with the larger methylated β-cyclodextrin cavity.

Retention of polyaromatic hydrocarbons

Fig. 5 shows the retention behaviour of the polyaromatic hydrocarbons on the methylated and unmodified cyclodextrin stationary phases in methanol-water (70:30). Because these solutes have no substituents, we need not consider the effect of substituents on the retention, and so the retention behaviour of these solutes may reflect their molecular sizes well.

Benzene has a retention time of more than 30 min on α-en-Su-Silica. The other five solutes are eluted quickly with similar retention times. These results strongly

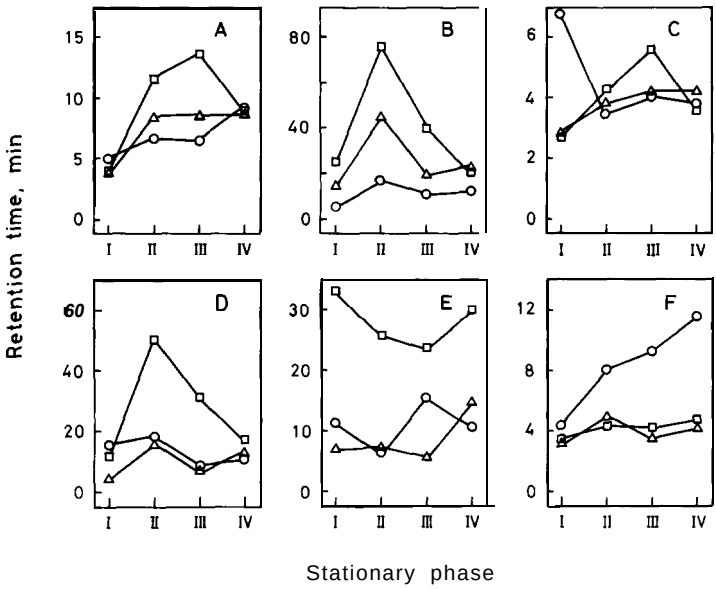

Fig. 4. Retention times of disubstituted benzene isomers (0 = ***ortho***; A = ***meta*** and ❑ = ***para***) on α-en-&r-Silica (I), Me-a-en&r-Silica (II), β-en-Su-Silica-(1) (III), and Me-β-en-Su-Silica (IV). Eluents: methanol-water (20:80) for I, III and IV except for IV-E (40:60); methanol-water (40:60) for II except for E (60:40). Solutes: A = cresol; B = iodoaniline; C = toluidine; D = nitroaniline; E = nitrophenol; F = dinitrobenzene.

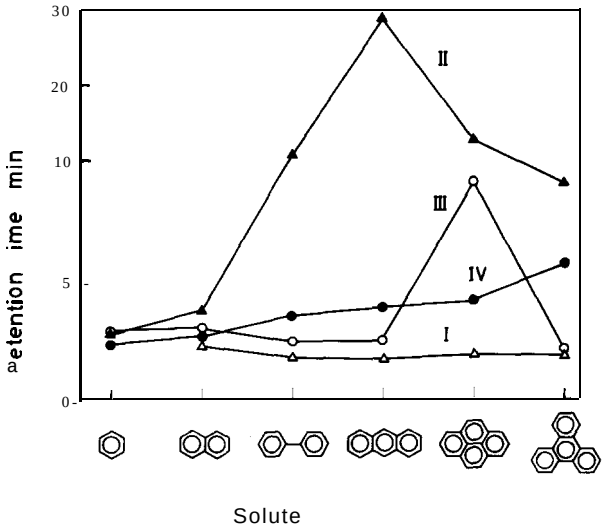

Fig. 5. Retention of polyaromatic hydrocarbons on a-en-Su-Silica (I), Me-a-en-Sri-Silica (II), β-en-Su-Silica-(2) (III), and Me-β-en-Su-Silica (IV). Eluent, methanol-water (70:30).

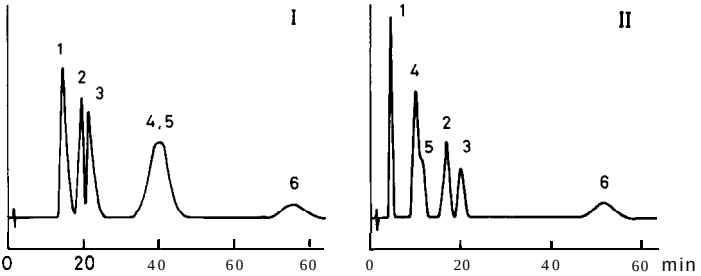

Fig. 6. Separation of mono- and disubstituted naphthalene derivatives on β-en-Su-Silica-(2) (I) and Me-β-en-Su-Silica (II). Eluent, methanol–1/15 Mphosphate buffer at pH 6.0 (30:70). Solutes: 1 = 1-naphthoic acid; 2 = 2-naphthol; 3 = 1-naphthol; 4 = 2-naphthoic acid; 5 = 6-hydroxy-2-naphthoic acid; 6 = 3-hydroxy-2-naphthoic acid.

suggest that these solutes, except benzene, cannot fit the unmodified a-cyclodextrin cavity well. The retention increases in the order benzene < naphthalene < biphenyl < anthracene on Me-a-en-Su-Silica. The longest molecule, anthracene, is retained most, and the wider molecules, pyrene and triphenylene, are eluted more quickly than anthracene. Pyrene can fit best into the unmodified β-cyclodextrin cavity. It is of great interest that the elution order of these polyaromatic hydrocarbons changes dramatically according to the cyclodextrin stationary phase used. These findings may give a clue to the size and depth of the methylated and unmodified cyclodextrin cavities.

Retention of naphthalene derivatives and drugs

Fig. 6 shows chromatograms of a mixture of mono- and disubstituted naphthalene derivatives on β-en-Su-Silica and Me-β-en-Su-Silica. On both the stationary

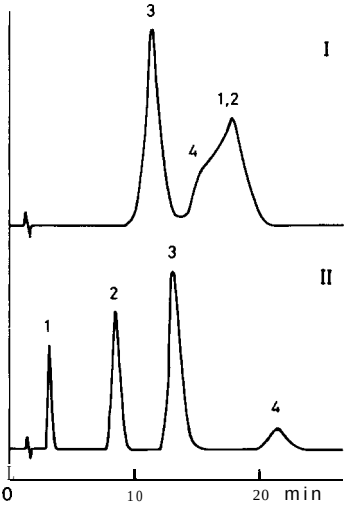

Fig. 7. Separation of antiepileptic drugs on β-en-Su-Silica-(2) in methanol-water (20:80) (I) and Me-β-en-Su-Silica in methanol-water (10:90) (II). Solutes: 1 = primidone; 2 = phenobarbital; 3 = carbamazepine; 4 = phenytoin.

phases, 2-naphthoic acid and 6-hydroxy-2-naphthoic acid cannot be separated. The methylated stationary phase retains l-and 2-naphthol more strongly than 2-naphthoic acid and its 6-hydroxyl derivative. The unmodified a-cyclodextrin stationary phase only weakly interacts with the solutes shown in Fig. 6 and does not separate them. 2-Naphthoic acid, 6-hydroxy-2-naphthoic acid and I-naphthol, or 2-naphthol and 3-hydroxy-2-naphthoic acid are not well separated on Me-a-en-&t-Silica, though retained strongly.

Fig. 7 gives another example of a remarkable change in selectivity after methylation. Primidone, phenobarbital and phenytoin cannot be separated on the unmodified β-cyclodextrin stationary phase, but they are separated completely on the methylated β-cyclodextrin stationary phase. A difference in the retention order of these drugs on the two phases is also apparent.

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