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LIQUID CHROMATOGRAPHY WITH CROWN ETHER-CONTAINING MOBILE PHASES

V*. EFFECT OF HYDROPHOBICITY AND CAVITY SIZE OF THE CROWN ETHER ON RETENTION OF AMINO COMPOUNDS IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The effects of the hydrophobicity and cavity size of crown ethers on the retention of several guest compounds (aromatic mono- and diamines) have been investigated in reversed-phase high-performance liquid chromatography. The resulting profiles of capacity factor, k' *vs.* crown ether concentration are discussed in terms of the hydrophobicity and cavity size of the crown ether and the stability of the host–guest complex formed between the crown ether and the amino group of a guest molecule. The capacity factors of the crown ethers themselves on a hydrocarbonaceous stationary ligand followed the sequence: 12-crown-4 < 15-crown-5 < 18-crown-6 $\ll$ dibenzo-30-crown-10 < dicyclohexyl-18-crown-6 < dicyclohexyl-24-crown-8 $\approx$ dicyclohexyl-27-crown-9 < dicyclohexyl-30-crown-10. The hydrophobicity of the crown ether was greatly enhanced by the presence of two cyclohexyl or two benzo substituents. The 18-membered crown ethers fit well a protonated primary amino group, resulting in an enhanced retention on the hydrocarbonaceous ligand, whereas 15- and 12-membered ones showed, respectively, weaker and almost no effects. The 24- and 27-membered crown ethers fit *o*-diamino compounds such as *o*-phenylenediamine and 2,3-diaminonaphthalene rather than monoamino compounds, suggesting that the two adjacent amino groups were simultaneously anchored to one large cavity, while the 30-membered crown ether fit snugly to 1,8-diaminonaphthalene rather than *o*-diamino compounds. The pH dependence of the retention enhancement is also discussed.

INTRODUCTION

In this series of papers[1–4] we have reported investigations on the host–guest interactions between crown ethers contained in mobile phases and amino compounds

* For Part IV, see ref. 4.

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in reversed-phase high-performance liquid chromatography (RP-HPLC). Since the hydration of a protonated amino group in the aqueous mobile phase is expected to become weak upon complex formation with the crown ether, the capacity factor of the guest on a hydrocarbonaceous stationary ligand increases with the hydrophobicity of the crown ether and the stability of the complex. Thus, it is possible to separate amino compounds on the basis of their structures, in contrast to conventional ion-pair chromatography where ionic interaction with a counter ion may occur less specifically. In addition, the ion-exclusion effect acting on the crown ether-associated ammonium ion, depending on the pH of the mobile phase, enables specific elution to be accomplished in a relatively short time. Obviously, the combined use of a crown ether and an ion-pairing reagent can be employed further to manipulate the specificity. Recent developments in the use of crown ethers in liquid chromatography have been reviewed[5–7]. The present paper deals with the effects of the hydrophobicity and cavity size of the crown ether on the retention of mono- and diamino compounds.

EXPERIMENTAL

Reagents and materials

The mono- and diamino compounds used as the guest materials were commercially available products of reagent grade, used without further purification. Benzylamine, 2,3- and 1,5-diaminonaphthalenes were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Benzoic acid, which was used as a reference material having no interaction with the crown ether, *o*-toluidine, 1,8-diaminonaphthalene, *o*-, m- and *p*-phenylenediamines were obtained from Nakarai Chemicals (Kyoto, Japan), aniline and phenylalanine from Wako Pure Chemicals (Osaka, Japan) and ampicillin was a clinical product from Takeda Pharmaceutical Industries (Osaka, Japan). 12-Crown-4 (12-C-4), 15-crown-5 (15-C-5), 18-crown-6 (18-C-6), dicyclohexyl-18-crown-6 (DC-18-C-6) and dicyclohexyl-24-crown-8 (DC-24-C-8) were purchased from Nakarai Chemicals, and dicyclohexyl-27-crown-9 (DC-27-C-9) and dicyclohexyl-30-crown-10 (DC-30-C-10) from Parish Chemical (Orem, UT, U.S.A.). Dibenzo-30-crown-10 (DB-30-C-10) was synthesized in this laboratory according to the established method[8]. The purity and molecular weight of the crown ethers were confirmed by HPLC and by fast atom bombardment mass spectrometry, respectively. Deionized water and methanol, both distilled from glass apparatus, were used to prepare the mobile phases.

Liquid chromatography

A liquid chromatograph equipped with a refractive index detector or a variable wavelength UV detector was used for the measurements of the capacity factors of the amino compounds and crown ethers. The operating conditions are given in Table I.

The guest materials were dissolved in a small portion of the mobile phase, and the minimum amount required for the detection was used in order to maintain linearity of the chromatographic system. The capacity factor was calculated as $(t_R - t_0)/t_0$, where t_R is the retention time of a solute at the peak in the elution curve, averaged over repeated measurements, and t_0 is that of a non-adsorbed substance (sodium nitrite).

TABLE I

HPLC CONDITIONS

Column temperature: 30°C. Mobile phase pH adjusted by hydrochloric acid.

Experiment	*Stationary phase*	*Mobile phase (water–methanol)*		*Detection*	*Data given in*
		Ratio	*Flow-rate*		
k' of crown ether	Ultrasphere C_8 (15 cm × 4.6 mm I.D., 5 μm)	1:2 (pH 2.5)	0.7 ml/min	RI	Table IIb
	LiChrosorb RP-18 (25 cm × 4.6 mm I.D., 10 μm)	65:35 (pH 2.5)	0.8 ml/min		Table IIa
k' vs. crown ether concentration	MBC-ODS (Shimadzu) (25 cm × 1.0 mm I.D.)	65:35 (pH 2.5)	30 μl/min	UV 220 nm	Fig. 1a,b Fig. 3a,b
		40:60 (pH 2.5)	60 μl/min		Fig. 4a,b Fig. 5 Fig. 7a,b Fig. 9a,b
		25:75 (pH 2.5)	60 μl/min		Fig. 6
k' vs. pH		40:60 (pH 2.0–4.3)	60 μl/min		Fig. 10

Data processing and plotting were carried out with a microcomputer (PC-9801, NEC).

RESULTS AND DISCUSSION

Capacity factor of crown ethers

The ability of a crown ether dissolved in the mobile phase to anchor an amino compound onto a hydrocarbonaceous stationary phase depends on the stability of the complex and the hydrophobicity of the crown ether. The relative hydrophobicity is reflected in the degree of retention of the crown ether itself on the same stationary ligand. The results given in Table II indicate that the capacity factor increased with

TABLE II

CAPACITY FACTORS OF CROWN ETHERS

For HPLC conditions, see Table I.

a		*b*	
Crown ether	*k'*	*Crown ether*	*k'*
12-C-4	1.15	18-C-6	0.26
15-C-5	1.55	DC-18-C-6	4.14
18-C-6	1.84	DC-24-C-8	4.55
		DC-27-C-9	4.52
		DC-30-C-10	4.76
		DB-30-C-10	2.26

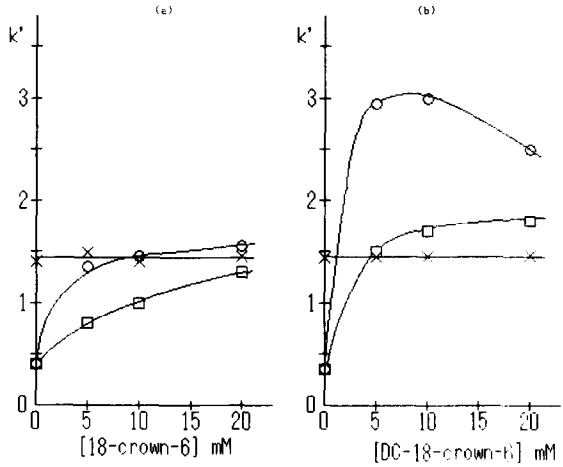


Fig. 1. Dependence of the capacity factors of ampicillin (○), phenylalanine (□) and benzoic acid (×) on the concentrations of 18-crown-6(a) and DC-18-crown-6(b) in the mobile phase.

increasing size of the crown ether cavity (except for DB-30-C-10), and a much greater increase resulted for the dibenzo or dicyclohexyl derivatives of the crown ethers. The capacity factors of these crown ethers having a wide range of hydrophobicities were, therefore, measured under different conditions, using 18-C-6 as a reference. The observed capacity factors follow the order: 12-C-4 < 15-C-5 < 18-C-6 ≪ DB-30-C-10 < DC-18-C-6 < DC-24-C-8 ≈ DC-27-C-9 < DC-30-C-10.

Effects of hydrophobicity and stability

It is known that the addition of a crown ether to the mobile phase in reversed-phase liquid chromatography has two opposite effects on the retention of amino compounds[2]. One effect is to increase the capacity factor by complex formation between the crown ether and protonated amino group, and the other is to decrease the capacity factor by competing with the free amino compound for binding to the hydrophobic stationary ligand. Hence, the apparent change in the capacity factor depends on relative strength of these effects. Fig. 1a and b shows, respectively, plots of k' *vs.* 18-C-6 concentration and k' *vs.* DC-18-C-6 concentration for ampicillin, phenylalanine and benzoic acid, the pH of the mobile phase being maintained at 2.5 by addition of hydrochloric acid. The capacity factor of benzoic acid remained almost unchanged despite the presence of 18-C-6 and DC-18-C-6, whereas those of ampicillin and phenylalanine increased significantly with the concentration of each crown ether. Further increase in the concentration of DC-18-C-6 caused the capacity factor of ampicillin to reach a maximum value at about 5–10 m*M* DC-18-C-6, where the two opposing effects mentioned above seem to be balanced, followed by a gradual decrease.

In general, the addition of a more hydrophobic crown ether gives rise to a greater initial increase in the capacity factor of an amino compound at a lower concentration in the mobile phase[2]. Fig. 2 demonstrates the effects of hydrophobicity, and complex stability on the k' *vs.* [C] profiles, where K_{LC} is the association constant of crown ether with a hydrocarbonaceous stationary ligand, and K_{CSH} is the stability

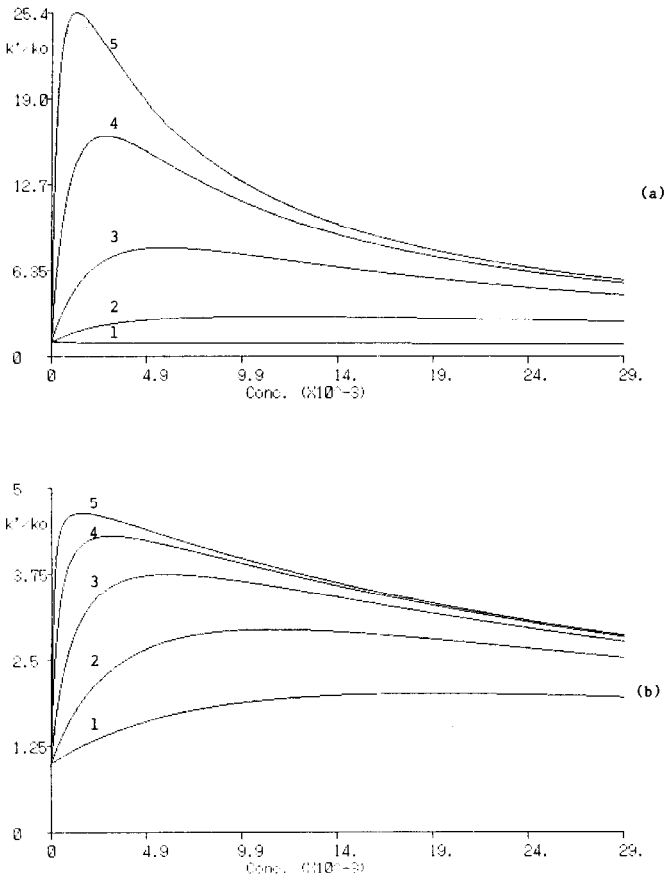


Fig. 2. Simulated curves of capacity factor against the concentration of crown ether in the mobile phase at various values of K_{LC} (M^{-1}) and K_{CSH} (M^{-1}). The simulation is based on the capacity factor equation derived from a chromatographic model of this particular system[2]. a, K_{CSH} = 200, K_{LC} = 5 (line 1), 25 (line 2), 125 (line 3), 600 (line 4), 3000 (line 5). b, K_{LC} = 25, K_{CSH} = 50 (line 1), 200 (line 2), 800 (line 3), 3200 (line 4), 12 800 (line 5).

constant of the complex between the crown ether and the amino compound. In drawing the curves in Fig. 2, various values of K_{LC} at a constant K_{CSH} (Fig. 2a) and various values of K_{CSH} at a constant K_{LC} (Fig. 2b) were substituted into the capacity factor equation which had been derived from a chromatographic model of this particular system (see eqn. 6 in ref. 2). The constant values for K_{LC} and K_{CSH} and the values of other parameters in the equation were chosen by referring to experimental data obtained previously[2]. Fig. 2a shows that an increase in K_{LC} at constant K_{CSH} gives rise to a marked increase in the maximum value of the capacity factor, $k'_{max.}$, whereas the crown ether concentration at the maximum, $[C]_{max.}$, is only slightly affected. In contrast, an increase in K_{CSH} at constant K_{LC} (Fig. 2b) results in a smaller increase in $k'_{max.}$ together with an appreciable decrease in $[C]_{max.}$. It is natural that the $k'_{max.}$ value observed at constant K_{LC} (Fig. 2b) reaches an upper limit at infinite increase in K_{CSH}.

These results would predict, in general, that the addition of an hydrophobic

crown ether, *e.g.*, DC-18-C-6, which forms a stable complex with an amino group of a guest molecule results in a large increase in the capacity factor at low concentration, and the addition of a less hydrophobic crown ether, *e.g.*, 18-C-6, which forms a stable complex brings about a large increase in capacity factor at higher concentration. On the contrary, the addition of an hydrophobic crown ether, *e.g.*, DC-24-C-8, which forms a less stable complex gives rise to smaller increase in capacity factor at low concentration, followed by a significant decrease at higher concentration. Obviously, the addition of a hydrophilic crown ether, *e.g.*, 12-C-4 and 15-C-5, which does not form a stable complex produces no apparent change in the capacity factor. Thus, it follows that the gentle increase in k' of phenylalanine without an apparent maximum (Fig. 1b) results from the lower stability of the complex formed with DC-18-C-6 compared to that of the ampicillin complex with DC-18-C-6.

Effect of cavity size of crown ether

Monoamino compounds. It is known that a protonated primary amino group fits best into the cavity of an 18-membered crown ether, where the ion–dipole interaction and hydrogen bonding play an important rôle in complex formation. The cavity size of the crown ether used in the mobile phase, therefore, is expected to be one of the factors that affect the retention of the amino compound on the hydrophobic stationary ligand . The effects of cavity sizes ranging from 12- to 30-membered rings on the changes in the capacity factors of several amino compounds are shown in Figs. 3 and 4. With 12- to 18-membered crown ethers, the degree of enhancement in capacity factor for ampicilline (Fig. 3a) and benzylamine (Fig. 3b) followed the sequence of cavity size; the addition of 12-C-4 (<20 mM) to the mobile phase brought about almost no apparent change in k', while 15-C-5 caused a slight increase and 18-C-6 gave rise to a much larger increase. The difference in the effects of 18-C-6 and DC-18-C-6 (Fig. 3a) is obviously due to the difference in their hydrophobicities rather than to the difference in cavity sizes. The results in Fig. 4a and b

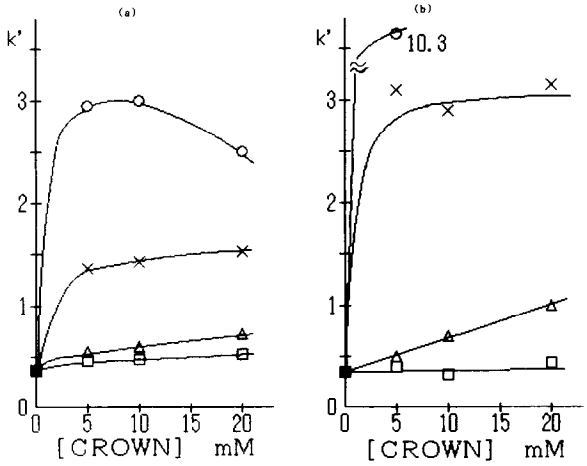


Fig. 3. Dependence of the capacity factors of ampicillin (a) and benzylamine (b) on the concentration of crown ethers in the mobile phase. O—O, DC-18-crown-6; ×—×, 18-crown-6; △—△, 15-crown-5; □—□, 12-crown-4.

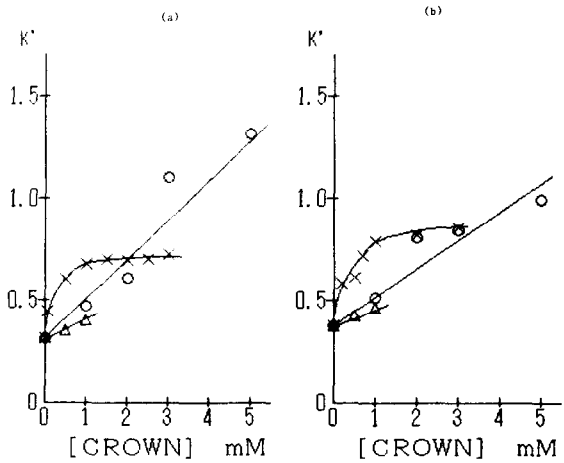


Fig. 4. Dependence of the capacity factors of aniline (a) and *o*-toluidine (b) on the concentration of crown ethers. O—O, 18-crown-6; × — ×, DC-24-crown-8; △—△, DB-30-crown-10.

indicate that the capacity factors of aniline and *o*-toluidine are increased more by addition of 18-C-6 (>3 m*M*) than by DC-24-C-8 and DB-30-C-10, although the solubility of the crown ether in the mobile phase limited the concentration to lower than 1.5 m*M* for DB-30-C-10 and 3 m*M* for DC-24-C-8. This suggests that these guest compounds form more stable and hydrophilic complexes with 18-C-6 than with the other crown ethers. Thus, it follows that the cavity sizes of 12-C-4 and 15-C-5 are too small and those of DC-24-C-8, DC-27-C-9, DC-30-C-10 and DB-30-C-10 are

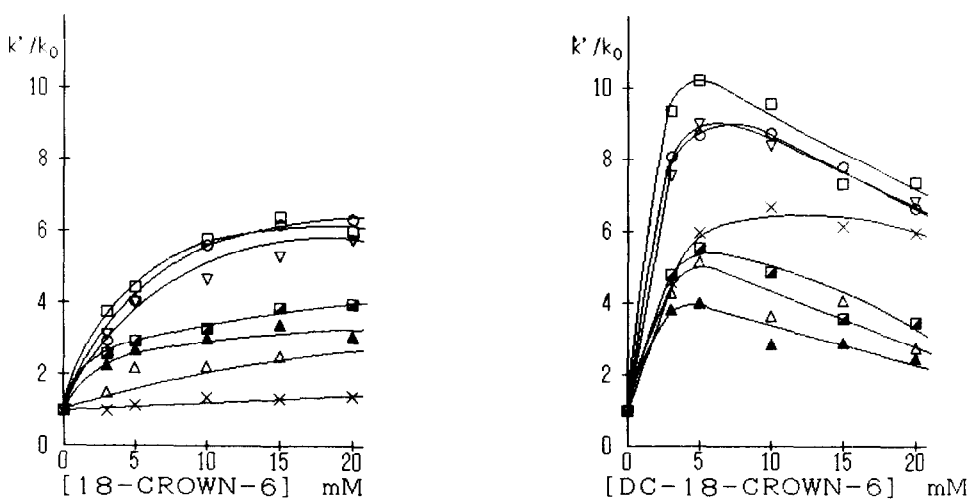


Fig. 5. Dependence of the capacity factors of diamino compounds on the concentrations of 18-crown-6 in the mobile phase. □—□, aniline; O—O, 2,3-diaminonaphthalene; ▽—▽, *o*-phenylenediamine; ◪—◪, *m*-phenylenediamine; ▲—▲, *p*-phenylenediamine; △—△, 1,5-diaminonaphthalene; ×—×, 1,8-diaminonaphthalene.

Fig. 6. Dependence of the capacity factors of diamino compounds on the concentrations of DC-18-crown-6 in the mobile phase. For symbols, see Fig. 5.

too large to form stable complexes with the guest amino group. The former crown ethers are not so hydrophobic as to compete with the guest compound in binding to the hydrophobic stationary ligand, so that their addition did not bring about an apparent change in the capacity factors. On the contrary, crown ethers of large cavity size and with two benzo or two cyclohexyl substituents are so hydrophobic that their addition to the mobile phase caused the capacity factor to exhibit a small maximum value at a low concentration, followed by an apparent plateau. The latter level is naturally lower than that attained with 18-C-6, because of low stability.

Diamino compounds. The changes in the capacity factors (k'/k_0) of diamino compounds upon addition of crown ethers in the mobile phase are shown in Figs. 5 and 6, where k_0 is the capacity factor in the absence of crown ether, *i.e.*, in the usual reversed-phase mode. Fig. 5 shows plots of k'/k_0 *vs.* 18-C-6 concentration for several diamino compounds, using aniline as a reference. The results indicate that the capacity factors of the diamino compounds are all smaller than that of aniline, and the capacity factors of *o*-diamino compounds (2,3-diaminonaphthalene, *o*-phenylenediamine) are increased more than those of other positional isomers. A similar trend is also found with DC-18-C-6 (Fig. 6) which, however, is so hydrophobic that the curves exhibit a maximum, and the maximum increases in k'/k_0 are higher than with 18-C-6 even at a low concentration in the mobile phase.

The plots of k'/k_0 *vs.* crown ether concentration with DC-24-C-8 and DC-27-C-9 are shown in Fig. 7a and b, respectively. DC-24-C-8 and DC-27-C-9 exhibit weaker interactions with the guest compounds than do 18-membered crown ethers (Figs. 5 and 6), and the addition of DC-24-C-8 causes a greater increase in the capacity factors of *o*-diamino compounds than for other positional isomers and aniline. Chromatograms for diaminonaphthalene isomers, in the absence and presence of DC-24-C-8, are shown in Fig. 8. The interaction between DC-24-C-8 and the *o*-diamino group is found to be responsible for the specific retardation of 2,3-diaminonaphthalene. These results suggest that a cavity size as large as a 24- or 27-mem-

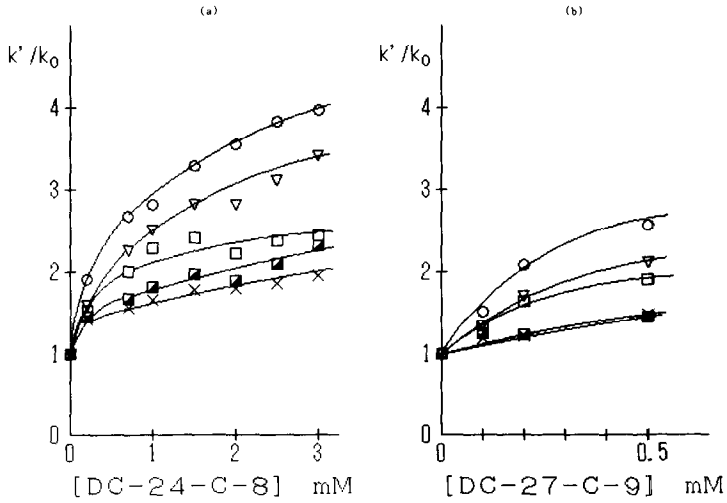


Fig. 7. Dependence of the capacity factors of diamino compounds on the concentrations of DC-24-crown-8 (a) and DC-27-crown-9 (b) in the mobile phase. For symbols, see Fig. 5.

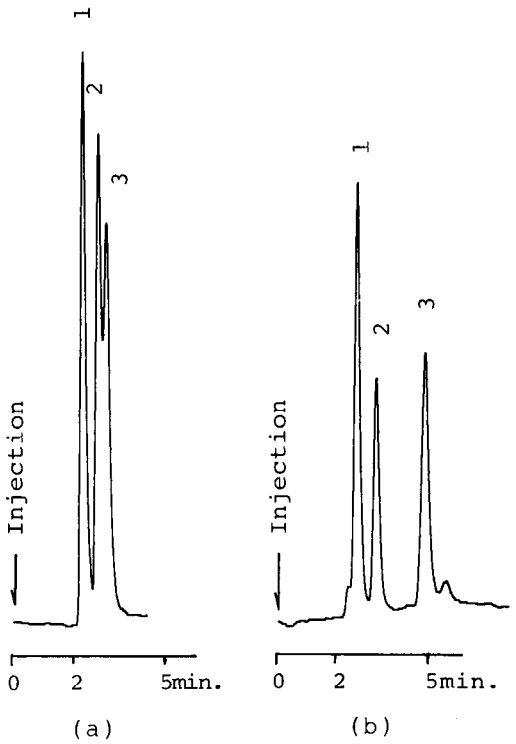
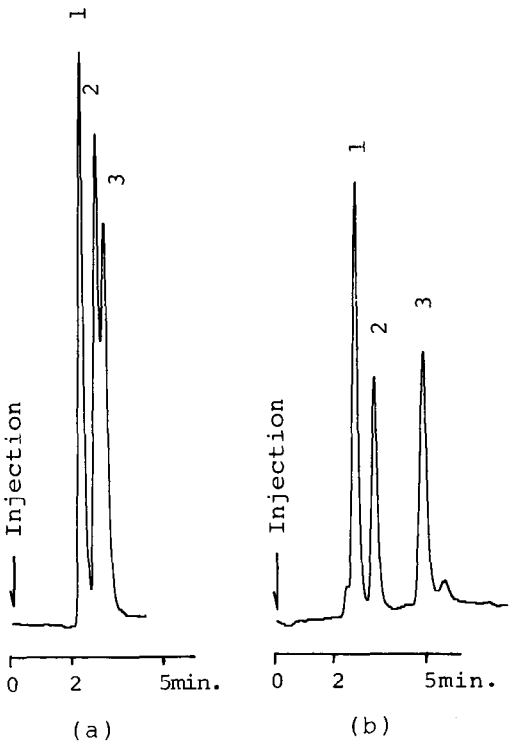


Fig. 8. Chromatograms of diaminonaphthalene isomers. Peaks: 1 = 1,5-diaminonaphthalene; 2 = 1,8-diaminonaphthalene; 3 = 2,3-diaminonaphthalene. Mobile phases: (a) water–methanol (1:1), pH 2.5 adjusted by hydrochloric acid; (b) as in (a) but containing 5 m*M* DC-24-C-8. Flow-rate: 0.7 ml/min. Stationary phase: Chemcosorb 5-ODSH (15 cm × 4.6 mm I.D.; Chemco, Osaka, Japan).

bered ring provides better conformity with *o*-diamino compounds than with others. One can thus speculate that two neighbouring amino groups which possibly form a univalent cation by sharing one proton could be simultaneously anchored to one large cavity, since either one of these amino groups can be protonated at a mobile phase pH of 2.5. The k'/k_0 values obtained with DC-27-C-9 are almost equal to those with DC-24-C-8 over a limited range of concentration (<0.5 m*M*). This is because these crown ethers have almost the same capacity factor (see Table II) and similar affinity for amino groups. Fig. 9a and b shows the plots of k'/k_0 *vs.* crown ether concentration obtained with DC-30-C-10 and DB-30-C-10. The addition of DC-30-C-10 or DB-30-C-10 to the mobile phase produced less enhancement in capacity factor than did DC-24-C-8 and DC-27-C-9 over a wide range of crown ether concentrations. This is possibly because of the weaker hydrophobicity and lower stability of the complex formed. It is interesting that the capacity factor of 1,8-diaminonaphthalene is increased more by addition of DB-30-C-10 than is 2,3-diaminonaphthalene. This sequence is the opposite of those observed with DC-24-C-8, DC-27-C-9 and DC-30-C-10 (see Figs. 7a,b and 9a), suggesting that factors other than the geometric fit between the crown ether cavity and the protonated amino group can affect the stability of the complex. One such factor could be the steric effect upon the interaction between the substituent groups in the host and guest molecules.



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The present investigation has demonstrated that the hydrophobicity and cavity size of the crown ether dissolved in the mobile phase affect the retention of amino compounds on the hydrophobic stationary ligand. This enables a new method for the specific separation of amino compounds by reversed-phase HPLC. Especially, the structure recognition due to the host–guest interactionn between the crown ether and the amino group in a guest molecule may be of value in the chromatography of biologically important materials bearing amino groups. The retention characteristics of amino acids, peptides and proteins in this system are being investigated.

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