

Selectivity Enhancement for *trans*-2-(2,3-Anthracenedicarboximido)cyclohexane-derived Diastereomers in HPLC by Using an Ordered Organic Stationary Phase

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2-(2,3-Anthracenedicarboximide)cyclohexane derivatives (AC) have been known as the evolutionary diastereomerizing reagents for enantiomer discrimination in HPLC with ODS]. However, a substantial separation of diastereomers can be observed only at lower temperatures, such as -40°C . Therefore, in this work, poly(octadecyl acrylate)-grafted silica, ODA_n was applied as an alternative stationary phase to ODS for the separation of AC-derived diastereomers. As a result, complete separation was achieved even under the conventional condition: for example, methanol as the mobile phase and 0°C as the column temperature. An investigation on the temperature dependency of the selectivity demonstrated that ODA_n shows a remarkable increase in selectivity at temperatures below 30°C , which almost agreed with the peak-top temperature of the endothermic peak in a DSC thermogram for ODA₃₅ immersed in a mobile phase. The better separation would be derived from a highly ordered structure of ODA_n and a carbonyl- π interaction with AC-derived diastereomers.

(Received November 13, 2006; Accepted January 29, 2007; Published March 10, 2007)

Introduction

It is well known that one enantiomer shows different biological activity from the other. For example, studies by Mori on the relationship between optical purity and biological activity of insect pheromones revealed that the biological activities were dramatically changed by their optical purities.¹⁻³ To determine an optical purity, a diastereomerizing method has been widely used, and thus many diastereomerizing reagents have been developed. However, this method has a serious problem in that it is very difficult, or even impossible, to discriminate diastereomers having chiral centers separated by more than four bonds. In order to solve this problem, Ohrui *et al.* developed chiral labeling reagents, 2-(2,3-anthracenedicarboximide)cyclohexane carboxylic acid and 2-(2,3-anthracenedicarboximide)cyclohexanol (AC).⁴⁻⁶ The use of these reagents made it possible to separate branched fatty acids or alcohols having a branched methyl group by reversed-phase HPLC with ODS.^{7,8} However, it was necessary to apply low column-temperature conditions (around -40°C) for their separation because the mobility of octadecyl groups in ODS and/or AC-derived diastereomer should be reduced.

On the other hand, we also developed a new packing material, comb-shaped polymer-grafted silica (ODA_n), for reversed-phase HPLC.^{9,10} This polymeric phase can be characterized by the facts that it can undergo a distinct phase transition between ordered

and disordered states, and it is in an ordered state at room temperature (Fig. 1). This indicates that the mobility of *n*-alkyl chains in the polymeric phase is much more reduced than those in ODS,^{11,12} and thus it is assumed that an ODA_n column would be effective for diastereomer separation, even under conventional temperature conditions. In this communication, we report that an enhancement of selectivity for 2-(2,3-anthracenedicarboximide)-cyclohexane-diastereomers can be achieved by using ODA_n as a stationary phase in reversed-phase HPLC.

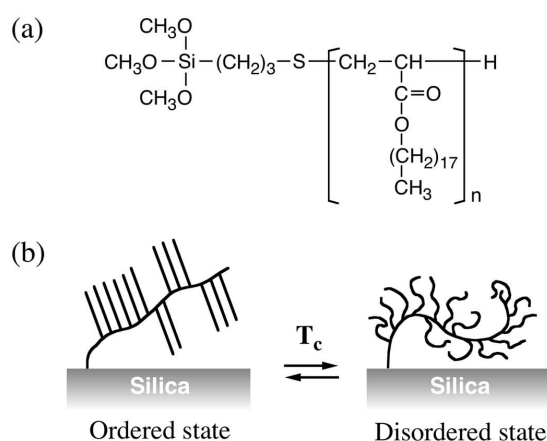


Fig. 1 Chemical structure of a comb-shaped polymer (a) and a schematic illustration of the phase transition of ODA_n (b).

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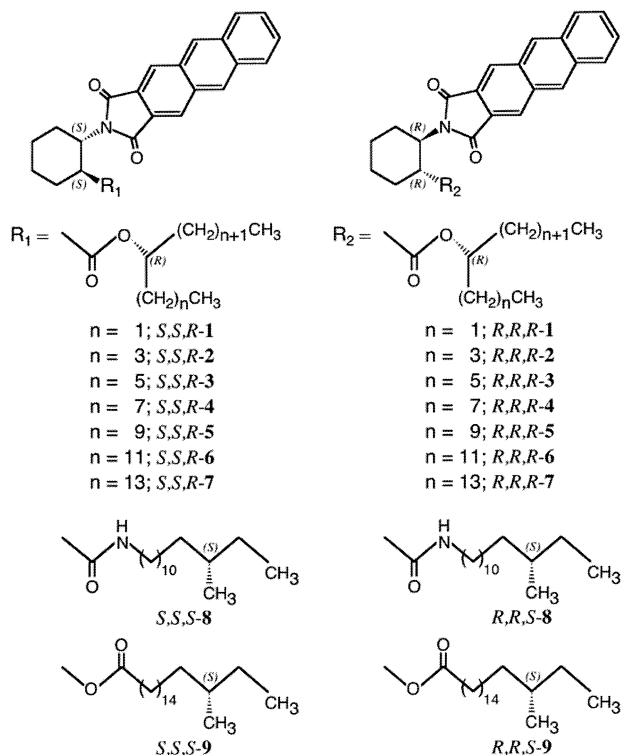


Fig. 2 Chemical structures of *trans*-2-(2,3-naphthalenedicarboximide)cyclohexane-derived diastereomers.

Experimental

Materials

Octadecyl acrylate and methyl acrylate were purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). 3-Mercaptopropyltrimethoxysilane was purchased from Azmax Co. (Chiba, Japan). YMC silica gel (120-5S, diameter 5 μm , pore size 120 \AA , specific surface area 330 $\text{cm}^2 \text{g}^{-1}$) was used as a porous silica.

Poly(octadecyl acrylate) with a terminal reactive group was prepared according to a previously reported telomerization method.¹⁰ The average degree of polymerization was determined by $^1\text{H-NMR}$ spectroscopy to be 35. The polymer was readily grafted onto porous silica by mixing in toluene at the reflux temperature. The amount of the immobilized polymer was determined by elemental analysis to be 17.5 wt%. Polymer-grafted silica (ODA₃₅; average degree of polymerization of 35) was packed into a stainless-steel column (4.6 mm i.d. \times 150 mm). Poly(methyl acrylate)-grafted silica (MA_{*n*}, *n* = 27) was prepared according to a similar procedure, 10 and then packed into a stainless-steel column (4.6 mm i.d. \times 250 mm). ODS (Inertsil ODS-3, 4.6 mm i.d. \times 250 mm, GL-Science, Inc., Tokyo, Japan) was used as a reference column.

For HPLC analysis, nine pairs of diastereomers were synthesized from the corresponding secondary alcohol, anteoiso amine and anteoiso acid with 2-(2,3-anthracenedicarboximide)cyclohexane carboxylic acid or 2-(2,3-anthracenedicarboximide)cyclohexanol, according to a previously reported method.^{5,6} The chemical structures and their abbreviations are given in Fig. 2.

Measurements

The chromatograph included a JASCO 980 PU pump and a

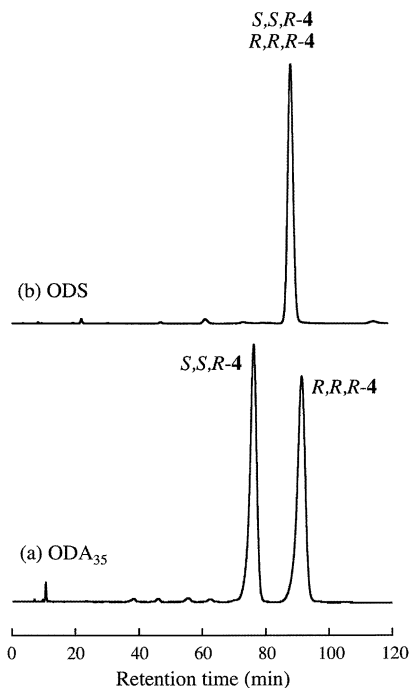


Fig. 3 Chromatograms for a mixture of *S,S,R-4* and *R,R,R-4* with ODA₃₅ (a) and ODS (b). Mobile phase, methanol at 0°C; flow rate, 0.5 mL min^{-1} ; detection, fluorescence detector (emission at 462 nm; excitation at 298 nm).

JASCO FP-2020 fluorescence detector (Jasco, Co., Tokyo, Japan). Five microliters of the sample were injected through a Reodyne Model 7125 injector. Liquid chromatography was carried out with a methanol, ethanol, mixture of ethanol-water or ethanol-tetrahydrofuran as mobile phase at a flow rate of 0.5 or 0.3 ml min^{-1} . Detection was performed by monitoring the fluorescence intensity at 462 nm (excitation at 298 nm). The column temperature was controlled in either a water/ethylene glycol jacket equipped with a RTE-111 instrument, or an ethanol bath equipped with a Cryocool CC100-II instrument (Thermo Neslab Instruments Inc., Newington, NH). The retention factor (*k*) was determined by $(t_e - t_0)/t_0$, where t_e and t_0 are the retention time of the samples and water, respectively. The separation factor (α) was given by the ratio of the retention factors. The resolution factor (R_s) was determined by $2(t_2 - t_1)/(W_1 + W_2)$, where t_2 and t_1 are the retention times of the samples, and W_1 and W_2 are the band-widths of the peaks. The asymmetry factor (A_s) was calculated from the ratio of the widths of the rear and front sides of the peak at 5% peak-height.

Thermograms by differential scanning calorimetry (DSC) of ODA₃₅ and their silica-supported derivatives were obtained at a heating rate of 1°C min^{-1} using EXTRA6000 with a DSC6200 (Seiko instruments, Inc., Chiba, Japan).

Results and Discussion

Comparison of diastereomer separation ability between ODA_{*n*} and ODS

To evaluate the diastereomer selectivity of ODA₃₅, the (1*S*,2*S*)- and (1*R*,2*R*)-AC esters of (*R*)-secondary alcohols (*S,S,R* and *R,R,R*, 1 - 7) were applied as solutes in HPLC. Since the *R,R,R* diastereomer is an enantiomer of the corresponding *S,S,S* one, they have the same chromatographic characteristics

Table 1 Retention and separation factors for (*R*)-secondary alcohols coupled with (1*S*, 2*S*)-or (1*R*, 2*R*)-AC

Diastereomer	Temperature/ °C	Mobile phase MeOH/EtOH/THF (v/v/v)	ODA ₃₅				ODS			
			<i>k</i> _{S,S,R}	<i>k</i> _{R,R,R}	α	<i>R</i> _s	<i>k</i> _{S,S,R}	<i>k</i> _{R,R,R}	α	<i>R</i> _s
1	0	100/0/0	1.36	1.52	1.12	1.15	1.99	1.92	1.04	0.56
	-20	100/0/0	3.02	3.59	1.19	2.20	2.86	2.75	1.04	0.53
	-40	0/100/0	2.16 ^a	2.58 ^a	1.20	0.87	1.24 ^a	1.27 ^a	1.02	0.13
2	0	100/0/0	2.16	2.66	1.23	2.63	3.52	3.50	1.01	0.11
	-20	100/0/0	5.52	7.50	1.36	4.13	5.60	5.71	1.02	0.35
	-40	0/100/0	3.08 ^a	4.24 ^a	1.38	1.71	2.16 ^a	2.42 ^a	1.12	0.75
3	0	100/0/0	5.45	7.50	1.38	5.50	7.11	7.18	1.01	0.21
	-20	100/0/0	20.58	33.71	1.64	7.32	13.86	14.88	1.07	1.42
	-40	0/100/0	11.37 ^a	19.31 ^a	1.70	3.23	5.89 ^a	7.93 ^a	1.35	2.08
4	0	100/0/0	19.27	23.63	1.23	3.55	15.20	15.17	1.00	0.05
	-20	0/100/0	13.39	17.34	1.30	2.30	3.02	3.15	1.04	0.32
	-40	0/80/20	28.14	36.53	1.30	1.99	3.00	3.36	1.12	0.87
5	0	0/100/0	5.18	5.85	1.13	1.37	2.42	2.41	1.00	0.08
	-20	0/100/0	39.23	48.43	1.23	2.06	5.47	5.62	1.03	0.28
	-40	0/70/30	51.58	67.35	1.31	2.09	2.94	3.15	1.07	0.57
6	0	0/100/0	7.68	8.25	1.07	0.84	3.66	3.65	1.00	0.03
	-20	0/100/0	—	—	—	—	9.00	9.21	1.02	0.26
	-20	0/90/10	31.70	35.85	1.13	1.36	—	—	—	—
	-40	0/70/30	—	—	—	—	4.49	4.63	1.03	0.26
	-40	0/50/50	18.90	21.35	1.13	1.06	1.00	1.01	1.01	0.10
7	0	0/100/0	11.12	11.66	1.05	0.56	5.56	5.55	1.00	0.04
	-20	0/100/0	—	—	—	—	15.34	15.15	1.01	0.15
	-20	0/80/20	22.78	24.65	1.08	0.88	2.73	2.71	1.01	0.08
	-40	0/70/30	—	—	—	—	7.57	7.39	1.02	0.19
	-40	0/50/50	30.09	33.72	1.12	0.95	1.43	1.43	1.00	0.01

a. Flow-rate: 0.3 ml min⁻¹; 0.5 ml min⁻¹ for others.

Table 2 Retention and separation factors for (*S*)-anteiso amine and acid coupled with (1*S*, 2*S*)-or (1*R*, 2*R*)-AC

Diastereomer	Temperature/ °C	Mobile phase (v/v/v/v) H ₂ O/MeOH/EtOH/THF	ODA ₃₅				ODS			
			<i>k</i> _{S,S,S}	<i>k</i> _{R,R,S}	α	<i>R</i> _s	<i>k</i> _{S,S,S}	<i>k</i> _{R,R,S}	α	<i>R</i> _s
8	0	10/0/90/0	1.96	2.06	1.05	0.37	2.90	2.90	1.00	0.01
	-20	0/0/100/0	1.33	1.53	1.15	0.71	0.71	0.71	1.00	0.01
	-40	0/0/100/0	4.86 ^a	6.22 ^a	1.28	1.27	1.28 ^a	1.32 ^a	1.03	0.16
	-40	0/0/70/30	0.91	0.99	1.09	0.36	0.48	0.47	1.02	—
9	0	10/0/90/0	25.56	26.23	1.03	0.32	17.83	17.82	1.00	0.01
	-20	0/0/100/0	36.56	37.22	1.02	0.11	3.55	3.54	1.00	0.02
	-40	0/0/80/20	>80	>80	—	—	3.78	3.84	1.02	0.10
	-40	0/0/70/30	37.47	43.45	1.16	1.11	—	—	—	—

a. Flow-rate: 0.3 ml min⁻¹; 0.5 ml min⁻¹ for others.

in an achiral HPLC system. Therefore, the use of an *R,R,R* diastereomer provided chromatographic data for the *S,S,S* one in the present work. Figure 3 shows typical chromatograms for a mixture of *S,S,R-4* and *R,R,R-4* with ODA₃₅ and ODS at 0°C. No separation was observed in ODS. Even each injection showed almost no separation (*k*_{R,R,R} and *k*_{S,S,R} = 15.2, *R*_s = 0.05). On the contrary, complete separation with a better α value was observed in ODA₃₅ (α = 1.23, *R*_s = 3.55).

A higher selectivity in ODA₃₅ at 0°C was also observed for diastereomers of **1–3** and **5–7**, where the separation factors depended on the alkyl length of the residual secondary alcohols, as shown in Table 1. Unfortunately, no distinct separation was observed for the diastereomers of **6** and **7** at 0°C (*R*_s = 0.84 and 0.56, respectively). However, their selectivities clearly increased upon lowering the column temperature, and thus a substantial separation was achieved at -40°C (*R*_s = 1.06 and 0.95). On the other hand, ODS showed no separation, even at -40°C (*R*_s = 0.26 and 0.19). The better separation in ODA₃₅ at

-40°C could be also seen in the separation for AC diastereomers having an anteiso amine or acid, as shown in Table 2. Here, it should be concluded that ODA₃₅ provided much better separation with high selectivity for AC-derived diastereomers compared with ODS.

Temperature dependency of diastereomer selectivity

As mentioned above in the introductory part, ODA_n undergoes a thermally induced phase transition between ordered and disordered states on silica. Therefore, the temperature dependency of the retentivity and selectivity was investigated by using methanol as a mobile phase. Figure 4 shows plots of the retention factors for *R,R,R-4* against the temperatures with ODA₃₅, ODS, and MA₂₇ which has the same chemical structure as that of ODA_n, except for the side chain. It is obvious that the plot is more temperature dependent in ODA₃₅ than ODS and MA₂₇. Interestingly, ODA₃₅ showed a shorter retention than ODS at temperatures above 10°C, but longer retention at 0°C.

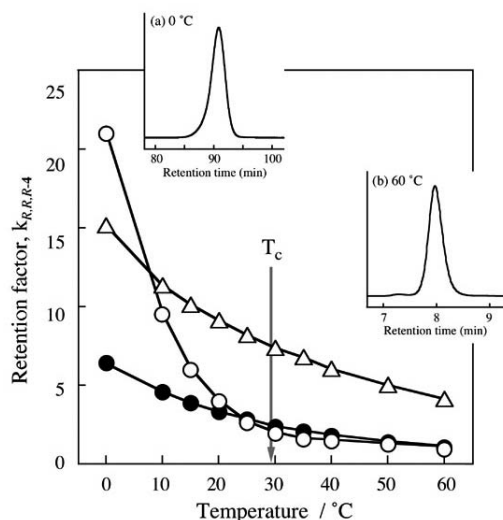


Fig. 4 Temperature dependencies of the retention factors for *R,R,R*-4 with ODA₃₅ (○), MA₂₇ (●) and ODS (△), and chromatograms for *R,R,R*-4 with ODA₃₅ at 0°C (a) and 60°C (b). Mobile phase, methanol at 0.5 mL min⁻¹. The arrow indicates the phase transition temperature (T_c) of ODA₃₅ dispersed in methanol.

This unusual retention increase was also observed for other diastereomers, as shown in Tables 1 and 2. For example, the retention factor for *R,R,R*-3 in ODA₃₅ was comparable with that in ODS at 0°C ($k = 7.50$ in ODA₃₅ and 7.18 in ODS), but a much higher retention factor was obtained in ODA₃₅ than ODS at -40°C ($k = 19.3$ in ODA₃₅ and 7.93 in ODS). A remarkable increase of the k values with decreasing temperature was accompanied by a change of the peak shape in the chromatogram. Peak-fronting was observed at 0°C, but not at 60°C, as shown in Fig. 4 ($A_s = 0.83$ at 0°C and 1.2 at 60°C). This difference is attributable to a disordered-to-ordered phase transition of the ODA₃₅ phase because the remarkable decrease of the A_s values was observed around 30°C, where the polymer phase undergoes a phase transition, as discussed later.

Figure 5 shows the temperature dependencies of the selectivity for *S,S,R*-4 and *R,R,R*-4. ODS provided almost constant and very small α values ($\alpha \sim 1.0$) in the temperature range of 0–60°C. On the other hand, the selectivity in ODA₃₅ was almost constant at 35–60°C ($\alpha = 0.99$ –1.01), but a remarkable increase in the selectivity was observed at temperatures below 30°C. In order to understand this distinct selectivity change of ODA₃₅, a DSC measurement was carried out for ODA₃₅ dispersed in methanol, which corresponded to a mobile phase used in the chromatography. The DSC thermogram showed an endothermic peak at 30°C as a peak-top temperature (T_c), which is indicated by arrows in Figs. 4 and 5. This temperature can be assigned to an ordered-to-disordered phase transition of the polymer phase immobilized on silica on the basis of the results of our previous investigation using a polarized microscopic observation¹⁰ and ¹³C-CP/MAS NMR spectroscopy.¹¹ The critical temperature of the selectivity change in Fig. 5 almost agreed with T_c of the polymer phase. This strongly suggests that the increasing selectivity with decreasing temperature is closely related to the phase transition of the polymeric phase, and the higher selectivity at 0–30°C can be realized by ordering of the polymer phase. A support for this estimation is the fact that no similar selectivity increase was observed in MA₂₇, which has no long-alkyl side chain, and thus undergoes no phase transition at 0–60°C. The further decrease

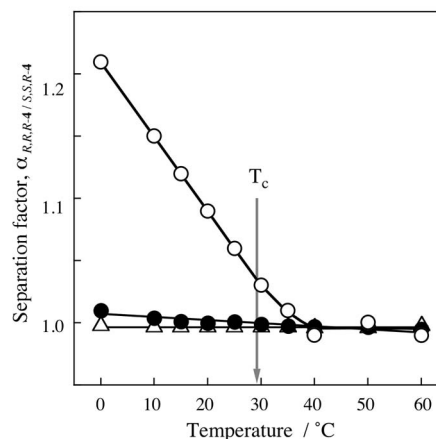


Fig. 5 Temperature dependencies of the separation factors between *S,S,R*-4 and *R,R,R*-4 with ODA₃₅ (○), MA₂₇ (●) and ODS (△). Mobile phase, methanol. The arrow indicates the phase transition temperature (T_c) of ODA₃₅ dispersed in methanol.

in the temperature enhances the selectivity of ODA₃₅, as shown in Tables 1 and 2. The higher selectivities were maintained even after addition of THF, which disturbed the molecular ordering of the polymer phase.

Discussion on high diastereomer selectivity

In our recent study, we reported that ODA_n, which is in an ordered state, showed a remarkable molecular-shape discrimination for polycyclic aromatic hydrocarbons (PAHs) in HPLC.^{9,10} Figure 6 shows a typical example for the separation of naphthalene and benz[*a*]anthracene with ODA₃₅ in a reversed-phase HPLC mode. Both of the compounds have the same number of carbon atoms, and thus their molecular hydrophobicities are very close to each other. Therefore, the separation factors with ODS were very small ($\alpha = 1.1$ –1.2), and there was no significant change at 0–60°C. On the contrary, ODA₃₅ showed much better selectivity ($\alpha = 1.4$ –2.1), especially at 0–30°C, and a critical temperature showing an increase of the selectivity was observed. This pattern is very similar to that observed in the diastereomer selectivity (Fig. 5).

We have also demonstrated that a carbonyl group of an acrylate moiety can work as an interaction source for an aromatic ring.^{13,14} This interaction is quite possible because our *ab initio* calculation for a formaldehyde-benzene complex as a model system proved that the carbonyl-benzene interaction (1.87 kcal mol⁻¹ at 2.9 Å)^{15,16} is more effective compared with CH₄-benzene (0.57 kcal mol⁻¹ at $R = 3.0$ Å)¹⁷ and benzene-benzene complexes (0.49 or 1.78 kcal mol⁻¹ at $R = 4.0$ or 2.5 Å).^{15,16} In addition, Hammett plots of the separation factors against the substituent effect in azobenzene derivatives as solutes showed a negative slope, indicating the fact that the carbonyl group in the ODA_n phase works as an electron-accepting group for π -electrons of an aromatic moiety as an electron-donating moiety.¹⁸ Based on these results, we have proposed a multiple carbonyl π -solute π interaction mechanism,¹⁵ as schematically illustrated in Fig. 6. It is certain that an ordered bonded phase can interact more effectively with a planar and linear compound (naphthalene) than with a planar and bending compound (benz[*a*]anthracene). Therefore, we assume that solute molecules are not incorporated into the ordered phase, but are rather adsorbed on the aligned carbonyl groups. The extremely large retention factors at -40°C (Table 1 and 2) may support the adsorption mechanism. On the other

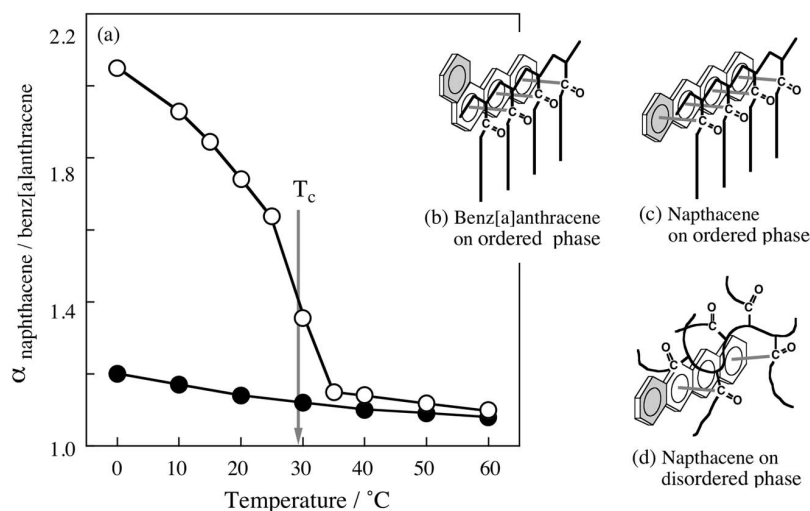


Fig. 6 Temperature dependencies (a) and proposal mechanism (b, c and d) for the separation factors between naphthalene and benz[a]anthracene with ODA₃₅ (○) and ODS (●). Mobile phase, methanol. If the carbonyl groups are linearly-aligned on a highly ordered structure, multiple interactions would be more effective for a linear compound, anthracene (c), than a bending compound, benz[a]anthracene (b). However, such shape selectivity disappears when the polymeric phase is in a disordered state (d).

hand, the disordered bonded phase does not have such specificity. The solutes can incorporate into the disordered bonded phase, and thus the separation mode is similar to that of ODS.

These understandings lead us to the following estimations for the good diastereomer selectivity in ODA₃₅: (1) we cannot neglect a partition effect derived from octadecyl groups of ODA₃₅ as one of the main driving forces for separation. However, (2) a carbonyl- π interaction is also included, and this subsidiary effect can be remarkably enhanced when ODA₃₅ is in an ordered state at a temperature below T_c . (3) This subsidiary effect can be brought about from an anthranil moiety of an AC diastereomerizing reagent. Ordering of the octadecyl groups in ODA₃₅ promotes a multiple interaction between the anthranil and carbonyl groups. (4) The diastereomer selectivity can be based on this multiple π - π interaction, because the selectivity cannot be detected when ODA₃₅ is in a disordered state.

Conclusions

In conclusion, we have demonstrated diastereomer separation with comb-shaped polymer-grafted silica, ODA_n instead of ODS. The use of ODA_n enables us to separate the 2-(2,3-anthracenedicarboximide)cyclohexane-derived diastereomers completely under the conventional condition: for example, methanol as a mobile phase and 0°C as a column temperature. This result would be derived from a highly ordered structure of ODA₃₅ and carbonyl- π interaction between ODA₃₅ and the diastereomers. The combinational use of 2-(2,3-anthracenedicarboximide)cyclohexane-derivatives and ODA_n should be powerful for enantiomer discrimination.

Acknowledgements

This work was partially supported by a Grant-in-Aid for Scientific Research of the Ministry for Education, Culture, Sports, Science and technology of Japan.

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