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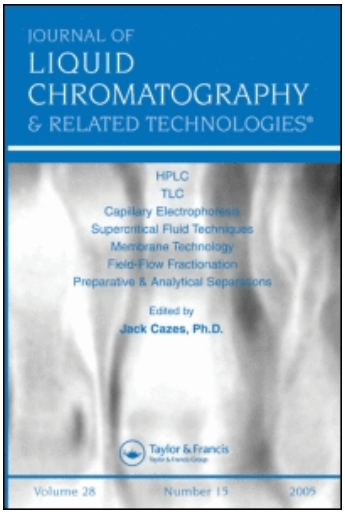

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Molecular Recognition of Acyclic Stereoisomer Using a Simple High Performance Liquid Chromatography. II

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MOLECULAR RECOGNITION OF ACYCLIC STEREOISOMER USING A SIMPLE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. II

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ABSTRACT

Chromatographic separation of acyclic stereoisomers of β-substituted alcohols in a reversed phase mode was studied. Conventional C_{18} and C_8 stationary phases and a specially designed 2-(1-pyrenyl)ethylsilylated silica gels (PYE stationary phase) were utilized for the separations. Since the β-substituted alcohols involve a hydroxy group and a hydrophilic functional group at β position to the hydroxy group, these possess a relatively tight intramolecular hydrogen bonding between these two hydrophilic functional groups to afford relatively fixed gauche conformation even in a aqueous mobile phase. In most cases, these diastereomeric β-substituted alcohols were resolved better on PYE stationary phase than on C_{18} and C_8 stationary phases. The elution order between erythro and threo isomers was inconsistent on C_{18} and C_8 stationary phases,

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when hydrophobic alkyl substituents were changed, on the other hand, threo isomer which was in more hydrophobic conformation was eluted faster on PYE phase when phenylsulfonyl group was included as a hydrophilic substituent. However, when an amide functional group was included as the hydrophilic substituent instead of the sulfonyl group, the elution order was reversed even on the PYE stationary phase, while the elution order was not changed on C_{18} stationary phase, when the alkyl substituents were equal. These findings may suggest that sterically plane PYE stationary phase can recognize a shape of stereoisomer rather than the difference in the hydrophobicity of solutes to give better resolution and the better relationship between the structure and the elution order than C_{18} and C_8 stationary phases. On the other hand, C_{18} and C_8 stationary phases recognize mostly the difference in the hydrophobicity of the solutes. Finally, for acyclic stereoisomers, some prediction to determine the configuration of stereoisomers utilizing a reversed phase mode can be made by the use of these stationary phases.

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INTRODUCTION

In the last decade, stationary phases such as alkylsilylated silica gels utilized for reversed phase high performance liquid chromatography (RPLC) has been improved and a lot of attractive stationary phases with unique concepts have been newly invented to become commercially available in most cases. The lack of chemical stability of silica based packing materials and undesired secondary retention mechanism based on residual silanol groups or metal impurities have been overcome by the use of a variety of technique [1] and recently, a majority of HPLC separation (> 70 %) takes place in a reversed phase mode. Especially, in bioscientific separation, aqueous separation condition is required and the growing need to the separation of biomolecules such as polypeptide and enzyme have been pushing a lot of newly designed stationary phase for reversed phase separations.

In a reversed phase mode, separation can be achieved mainly based on the difference in partition and/or adsorption of solutes between hydrophobic stationary phase and aqueous mobile phase which is due to the hydrophobic interaction [2], therefore it is

better method to separate the organic compounds which possess different hydrophobic alkyl substituents than that in a normal phase mode using silica gels as an adsorbent. From the view point of isomer separation, since plane adsorbents silica gels seem to recognize a molecular planarity better, traditionally, relatively simple silica gel column chromatography in a normal phase mode has been utilized to separate actual reaction product involving stereoisomers by a majority of organic synthetists. However, it has been occasionally known that it is relatively difficult to control resolution when the target compound is too hydrophobic or hydrophilic because in a normal phase mode, hydrogen bonding is one of the most important interactions for the separation [3].

A problem to utilize reversed phase packing materials to usual open column chromatography is the packing materials can be hardly packed into column using aqueous mobile phases because of their hydrophobic surface properties and this disadvantage may be preventing the use of a reversed phase chromatography in the actual preparative product separation using a simple and easy open column. Recently, the reversed phase packing materials for open column chromatography [4] are commercially available, therefore, the opportunity to apply separation in the reversed phase mode for actual preparative separation of synthetic mixtures will become higher. Moreover, In a reversed phase mode, relatively cheap mobile phase such as aqueous methanol or aqueous acetonitrile can be used. Therefore, in a near future, reversed-phase mode will become a main technique for the separation of a variety of compounds including sterically similar compounds such as stereoisomers.

As described in a previous paper [5], it is very important for a molecular recognition of stereoisomers to use the stationary phase which can recognize planarity of solutes. From this point of view, the separation of stereoisomers in the reversed phase mode seems to be unsuitable to this purpose because the difference in hydrophobicity of solutes has been thought to be a dominant retention mechanism in reversed phase liquid chromatograph (RPLC) [6]. Conventional C_{18} and C_8 stationary phases (Figure 1)

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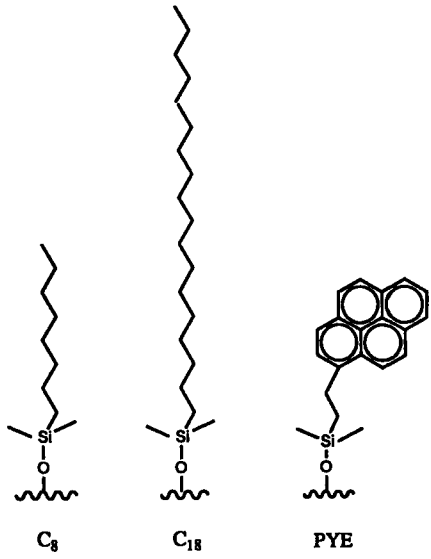

FIGURE 1. Stationary phases

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resolve solutes mainly based on a difference in their hydrophobicity, however, interestingly even sterically similar C_{18} and C_8 phases sometimes show a different selectivity to the same solutes [7]. This kind of unexpected phenomenon was explained based on a difference in the structure of stationary phase in a aqueous mobile phase. In addition to above interesting selectivity, specially designed stationary phase such as 2-(1-pyrenyl)ethylsilylated silica gels (PYE stationary phase) (Figure 1) show good resolution for mainly cyclic stereoisomers such as the disubstituted cyclohexanes [8]. Moreover, recently, another attempts were made for the molecular recognition of acyclic stereoisomers [9], therefore, a study in a molecular recognition of acyclic stereoisomers using conventional reversed phase stationary phases is worth investigating and will become very important and useful method as various studies will be made.

Since acyclic compounds have interested organic synthetists because of synthetic importance as described before [5], here we try to make a resolution of acyclic

stereoisomers by a simple RPLC and some attempts to determine and recognize the structures of acyclic stereoisomer based on the elution orders because the easily acceptable and cheap technique is really required for organic synthetists. Although some good chiral HPLC columns for a direct separation of enantiomers are now available, those are clearly more expensive than conventional reversed phase packing materials. So that, the separation of the diastereomer derived from the enantiomer on an achiral stationary phase are sill useful and important methods. Moreover, a very high diastereomeric ratio can be determined by HPLC, while NMR method can hardly determine that high ratio correctly as described in the our previous work [5].

From this point of view, β-substituted acyclic alcohols have been chosen as the stereoisomer applied in this primary work. β-substituted acyclic alcohols potentially include various substituents, therefore, this type of compound has been utilizing as an important intermediate for the syntheses of lots of naturally occurring compounds [10]. In addition, recently, enzymatic reductions of α-substituted carbonyl compounds are being watched with keen interest [11] and the measurement of diastereomeric ratio is also a very important factor to estimate the reaction pathway. Therefore it is also a good target for analytical chemists. Usually, the determinations of such diastereomeric isomers have been done using gas-liquid chromatographic method, however, thermal isomerization of compounds can not be negligible perfectly and preparative scale separation can hardly take place through gas-liquid chromatography.

Since a molecular interaction between ligand of stationary phase and solute molecules is an important retention mechanism, the separation of stereoisomers in a reversed phase mode is very interesting from the view point of molecular recognition by high performance liquid chromatography even if the interaction is weak.

EXPERIMENTAL

Materials : All β-substituted acyclic alcohols were prepared in our laboratory using a typical preparation method described elsewhere [12]. The configuration of

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diastereomers was determined by NMR techniques according to the reported method [13] after an isolation procedure of each diastereomer by a column chromatography.

All chromatographic grade solvents were purchased from Nacalai Tesque (Kyoto, Japan) and used without further purifications. Based silica gels were purchased from Nomura Chemical Inc., (Seto, Japan) and C_{18}, C_8, and PYE stationary phases were prepared in our laboratory using a typical modification technique [2] involving subsequent treatment with chlorotrimethylsilane for the further silylation namely "end capping". All stationary phases were packed into stainless-steel columns (4.6 mm I.D. X 150 mm) under a wet condition.

Equipments : Chromatography was carried out with a Jasco 880-PU intelligent HPLC pump equipped with a Rheodyne 7125 valve loop injector. Both UV detector of a Jasco UVIDEC-100-III and RI detector of a Waters Differential Refractometer R401 were used for peak monitor. Columns were thermostated at 30 ± 0.1 °C and all measurements using the same mobile phase were finished within the working time of a day because the conditions of mobile phase such as water content or exact mixing rate of binary solvent system must sensitively affect the elusion of samples. D_2O and/or uracil were utilized for the t_0 measurement and capacity factor k' was calculated based on the retention time determined using a Shimadzu C-R4A chromatopac. Reproducibility of data in duplicate was better than 1 %.

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RESULTS AND DISCUSSIONS

It is well known that aliphatic stationary phases (C_8 and C_{18}) and aromatic stationary phase (PYE) as illustrated in Figure 1 showed different retention selectivity in a reversed phase mode [7]. For example, aromatic stationary phases provide relatively long retention time for aromatic hydrocarbons, while aliphatic hydrocarbons are retained much longer on aliphatic stationary phases than that on aromatic stationary phases. These

findings may suggest a molecular recognition based on a preferable retention between stationary phase and functional groups as well as the hydrophobic interaction is also one of the important retention mechanisms in a reversed phase mode.

Generally, when a hydrophobicity of solutes involve some difference, the aliphatic stationary phases may resolve well even if solutes are in a diastereomeric relationship each other. For example, 1,4-disubstituted cyclohexanes were reported to be resolved better on C_{18} stationary phase than those on PYE stationary phase, while 1,2-disubstituted cyclohexanes tended to be separated better with much shorter retention time on PYE stationary phase. These different selectivities were explained based on the recognition of the structural planarity of 1,2-disubstituted cyclohexanes on PYE stationary phase, while based on the differences in the hydrophobicities of the solutes, C_{18} stationary phase gave better separation for 1,4-disubstituted compounds [8]. So that, appropriate combination of both stationary phases in a reversed phase mode is useful separation method for isomer separations.

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Cyclic isomers seem to be separated better even in a reversed phase mode because their conformations which result in the different hydrophobicity tend to be fixed because of the existence of rigid ring structure. On the contrary, acyclic stereoisomers involve relatively flexible conformation which may result in poor separation in the reversed phase mode. The stereoisomers utilized in this paper are β-substituted acyclic alcohols as illustrated in Figure 2. These compounds fortunately involve an intramolecular hydrogen bonding between the hydroxy group and the substituent (Z) which includes a hydrogen acceptor to afford relatively fixed gauche conformation by namely gauche effect [14] even in a solvent including methanol or acetonitrile. These conformations can be confirmed by the founded different coupling constant based on the fixed conformations as illustrated in Figure 3 [13].

Although PYE stationary phase possesses an ability of a recognition of structural planarity of solutes, the hydrophobicity of solute may be still important factor for the

FIGURE 2. β-Substituted acyclic alcohol

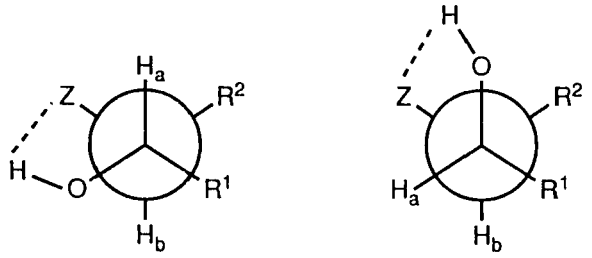

FIGURE 3. *Gauche* conformation of β-substituted acyclic alcohol

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retention. For this discussion, substituted ethanes with a variety of functional groups are utilized and their retention behavior on the tree stationary phases are summarized in Table 1. Actually, these values are separation factors (α) based on k' value of nitroethane which provides the shortest retention time within those solutes.

As expected, aliphatic stationary phases C_{18} and C_8 showed much longer retention time for hydrophobic compounds such as phenylpropane (No. 7) and phenylthio ethane (No. 6) than PYE stationary phase.

Interestingly, the selectivity for the amide derivative (No. 3) and the sulfonyl derivative (No. 4) are different between on aliphatic and aromatic stationary phases. The amide derivative was retained relatively longer on aliphatic stationary phase, while

TABLE 1. Retention Behavior of X-C_2H_5

No.	X-	$k'_{(X-)}/k'_{(nitro)}$		
		C_{18}[a)]	C_8[a)]	PYE[b)]
1	PhOOC-	5.63	6.03	4.20
2	NO_2-	1	1	1
3	PhNHOC-	2.14	2.42	1.84
4	$PhSO_2$-	1.09	1.67	3.18
5	PhSO-	1.50	1.48	3.73
6	PhS-	18.1	15.6	8.91
7	$PhCH_2$-	34.9	29.8	9.27
8	PhO-	10.3	8.03	6.03

a) Mobile phase 70 % methanol - 30 % water (v/v)
b) Mobile phase 60 % methanol - 40 % water (v/v)

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aromatic stationary phase (PYE) retained the sulfonyl derivative longer. These findings are mainly due to electronic characteristics of phenyl ring. On relatively electron rich PYE stationary phase, electron deficient π-acidic compound may be retained preferably. Therefore, the phenylsulfonyl derivative with an electron deficient phenyl ring was retained longer on PYE stationary phase [15]. In addition, the difference in retention selectivity for the amide derivative was smaller than that for the sulfonyl derivative. This point is basically similar to the selectivity found on silica gels in a normal phase mode [5].

For the separations of the diastereomers, β-substituted acyclic alcohols with phenylsulfonyl group as a substituent at β position (Figure 4) were examined on PYE and C_{18} stationary phases. These results are summarized in Table 2.

On C_{18} stationary phase, elution orders were inconsistent when the alkyl substituents were changed, on the other hand, threo isomers tended to be eluted faster on PYE stationary phase. Since PYE stationary phase recognizes mainly molecular planarity of solutes, above diastereomers may possess relatively similar and fixed conformation as described earlier in the chromatographic solvent system even if the alkyl substituents are changed. It is natural because the hydrophobicity of diastereomers should be changed

threo *erythro*

FIGURE 4. β-Substituted acyclic alcohol with phenylsulfonyl group

TABLE. 2 Separation of Diastereomers with Phenylsulfonyl Group

No.	R^1	R^2	k'(erythro)/k'(threo)		Mobile phase[a] (%)
			PYE	C_{18}	
1	CH_3	CH_3	1	0.79	45
2	CH_3	C_2H_5	1	1.10	45
3	CH_3	n-C_8H_{17}	1.10	1	85
4	n-C_3H_7	CH_3	1.15	0.90	60
5	n-C_3H_7	n-C_8H_{17}	1.15	1.07	85
6	i-C_4H_9	CH_3	1.37	0.91	60
7	i-C_4H_9	n-C_4H_9	1.20	1.08	80
8	i-C_4H_9	n-C_8H_{17}	1.20	1.12	85
9	n-C_8H_{17}	CH_3	1.23	1.00	80
10	n-C_9H_{19}	CH_3	1.23	1.03	85
11	n-C_9H_{19}	n-C_8H_{17}	1.14	1.09	100

a) These values mean volume % of methanol in water.

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d ıe to the different hydrophobic alkyl substituents, and on C_{18} phase, this difference in tl e hydrophobicity is the most dominant interaction for the molecular recognition. Ir terestingly, when the diastereomers included methyl and n-octyl substituents as R^1 or R^2 (No. 3 and 9), almost no separation was found on C_{18} stationary phase. Since a ıother diastereomers can be separated on C_{18} stationary phase, theses alkyl substituents n ay be a turning point in the balance of hydrophobicity. On the contrary, both d astereomers were resolved well on PYE stationary phase. The typical chromatograms o ' No. 3 on C_{18} and PYE stationary phases are depicted in Figure 5.

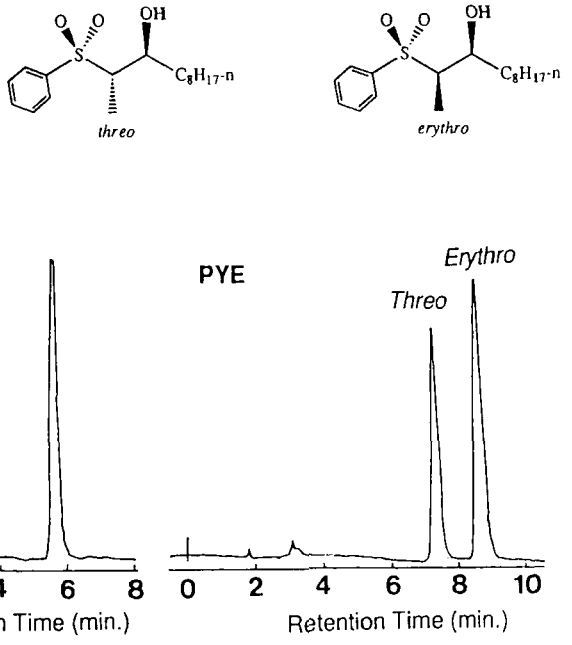

FIGURE 5. Separation of 2-phenylsulfonylundecane-3-ol in a reversed phase mode
Mobile phase; 80 % aqueous methanol
Flow rate; 1 ml/min.
Detection; UV 254 nm

These differences in the selectivity between on C_{18} and PYE stationary phases can be explained based on the difference in molecular planarity derived from their gauche conformations. As indicated in Figure 3, erythro isomer provides sterically more compact conformation than threo isomer because in threo isomer, hydrophobic substituents (both alkyl substituents) and hydrophilic substituents (the hydroxy and phenylsulfonyl group) are parted into the opposite direction each other to afford bulkier conformation. To the plane compound, PYE stationary phase usually shows preferable retention, so that, almost all diastereomers can be separated on PYE phase based on their difference in the sterical bulkiness. Based on this explanation, on PYE stationary phase,

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when alkyl substituent R^1 was equal (No. 6, 7, and 8) the longer or bulkier alkyl substituent R^2 which made erythro isomer bulkier afforded smaller separation factor, while bulkier alkyl substituent R^1 which inversely made threo isomer bulkier gave better separation if the alkyl substituent R^2 was the same (No. 1, 4, 6, 9, and 10).

On the other hand, C_{18} stationary phase separates these diastereomers based on the difference in the hydrophobicity, therefore, when one or both alkyl substituents are changed, a hydrophobic balance may be changed to result in the inversion of elusion orders. It is usually found that the hydrophobicity of an alkyl substituent closer to a hydrophilic functional group is compensated in a reversed phase mode than the same alkyl substituent exists apart. Based on this finding, the erythro isomer is more hydrophilic than the threo isomer because in erythro isomer, both hydrophilic and hydrophobic substituents are closer each other than those in threo isomer. In fact, when R^2 which is closer to the hydrophilic hydroxy group in erythro isomer than that in threo isomer involves less hydrophobic substituent (CH_3), the erythro isomer is eluted much faster than more hydrophobic threo isomer. However, in these cases (No. 1, 4, 6, 9, and 10), when the alkyl substituent R^1 becomes more hydrophobic, the separation factor becomes smaller because the balance of the hydrophobicity may be compensated to become very similar. In addition, when the alkyl substituent R^2 becomes more hydrophobic, erythro isomer is retained longer on C_{18} stationary phase (No. 1 and 2, 4 and 5, and 7 and 8). The separations are affected by the balance of the hydrophobicity of the diastereomers and the small change of the alkyl substituent may make a drastic change in a balance or the hydrophobicity as well as the elution order.

The separations of β-substituted alcohols with hydrophilic different functional group were also examined using an amide and a nitro derivatives compared with the phenylsulfonyl derivative as described at the previous section. Three kinds of diastereomers were employed as illustrated in Figure 6 and the results on three stationary phases are summarized in Table 3.

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threo *erythro*

FIGURE 6. 2-Substitutedundecane-3-ols with different hydrophilic functional groups

TABLE 3. Separation of Diastereomers

Z-	k'(k'$_{erythro}$/k'$_{threo}$)		
	PYE	C_{18}	C_8
NO_2-	1.77(e)	1.99(e)	
	(0.94)	**(0.94)**	1.32[a]
	1.88(t)	2.11(t)	
PhNHOC-	1.86(e)	2.74(e)	1.73(e)
	(0.93)	**(0.90)**	**(0.93)**
	2.01(t)	3.03(t)	1.87(t)
$PhSO_2$-	3.08(t)	2.28(e)	
	(1.23)	**(1.00)**	1.48[a]
	3.80(e)	2.29(t)	

(e) and (t) mean k' value of erythro and threo isomers, respectively.
a); In these cases, isomers were not separated on C_8 stationary phase.
Value in a bracket is separation factor (k'$_{erythro}$/k'$_{threo}$).

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On C_8 stationary phase, only the diastereomer including the amide functional group was separated. However, the separation factor is almost equal to that on PYE stationary phase. On C_{18} stationary phase, the erythro isomers were always eluted faster than the threo isomers. This may be due to the difference in the hydrophobicity between the erythro isomer and the threo isomer where the erythro isomer is less hydrophobic. As described earlier, although the change of the alkyl substituent affects the elution order very much and the elution order on C_{18} phase is inconsistent based on the type of diastereomer, the elution order may be decided by only the balance in the hydrophobicity

as expected in spite of the different hydrophilic functional groups when alkyl substituents which play an important role in the hydrophobicity of compound are equal.

Interestingly, on PYE stationary phase, the erythro isomers with the nitro and the amide functional groups were eluted faster than the threo isomers, however separation factors were smaller than that of the diastereomer with phenylsulfonyl functional group. As summarized in Table 1, the sulfonyl group was preferably retained on PYE stationary phase because of the electron deficient phenyl ring, on the other hand, the amide and nitro functional groups provided shorter retention time than the phenylsulfonyl group. In these cases, the amide and nitro functional groups act as a hydrophilic substituent more and molecular recognition may be determined by a balance in their hydrophobicity rather than a molecular planarity which is similar to those on C_{18} stationary phase. In fact, the retention time of the diastereomer including the phenylsulfonyl group is longer on PYE stationary phase than that on C_{18} stationary phase, however, the diastereomer including the amide functional group is retained longer on C_{18} stationary phase than that on PYE stationary phase. Of course both factors may concur, and even on PYE stationary phase the balance in the hydrophobicity is one of the dominant retention mechanism when the substituting functional group is too hydrophilic which results in much shorter retention time.

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As described in the previous paper, on silica gels in a normal phase mode, the elution order of the same diastereomer was completely inverse compared with on PYE stationary phase in a reversed phase mode. However, a type of molecular recognition on PYE stationary phase is very similar to those on silica gels which is based on the molecular planarity and the a specific retention to included some functional group. The specific retention on PYE stationary phase may be based on a electron characteristics as described in the previous section. An electron rich pyrene ring may interact more tightly with electron deficient aromatic ring. For example, PYE stationary phase showed longer retention time with phenylsulfonyl group where electron deficient phenyl ring was

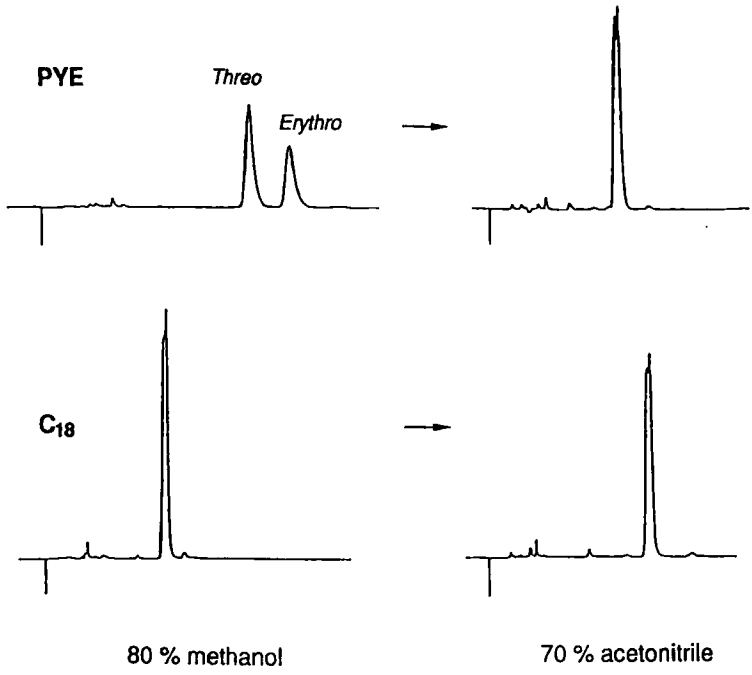

FIGURE 7. An effect of the mobile phase on the separation of the diastereomer
Sample; 2-phenylsulfonylundecane-3-ol
Flow rate; 1 ml/min.
Detection; UV 254 nm

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included, while amide functional group including relatively electron rich phenyl ring showed shorter retention time. Interestingly, when the mobile phase was changed from aqueous methanol to aqueous acetonitrile which can diminish π–π interaction of PYE stationary phase because of its π electron, on PYE stationary phase, the molecular recognition was completely lost, while on C_{18} stationary phase, no change was found (Figure 7). This finding may suggest the molecular recognition on PYE stationary phase involves π–π interaction between pyrene ring and functional group included in the solute diastereomer, while on C_{18} stationary phase, almost only hydrophobic interaction is the

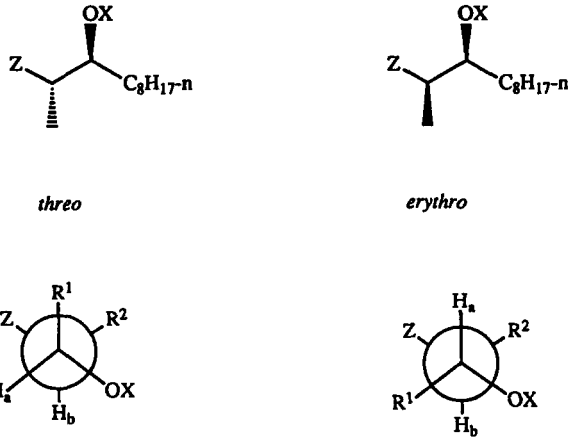

FIGURE 8. Protected alcohols and their anti conformation

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dominant retention mechanism. PYE stationary phase can recognize diastereomer when the same functional groups in included and there is almost no difference in the elution order by changing hydrophobic alkyl substituents. On the contrary, C_{18} stationary phase can recognize diastereomer when the same alkyl substituents are involved and the elution order is not changed with the different hydrophilic functional groups.

As described above, to the molecular recognition of acyclic β-substituted alcohols, the intramolecular hydrogen bonding plays an important role to fix the conformation. Here, an esterification or a silylation of the hydroxy group were employed to destroy the intramolecular hydrogen bonding. Actually, esterification by the use of a chiral carboxylic acid chloride such as α–methoxy-α-trifluoromethylphenyacetic acid (Mosher acid) is usually utilized for the determination of enatiomeric excess by NMR or HPLC [16]. Since β-substituted alcohols are already diastereomers, an achiral benzoyl chloride and chlorotrimethylsilane were utilized to the functionalizations. By these functionalizations the intramolecular hydrogen bonding may be destroyed because of the

TABLE 4. Separations of The Functionalized Alcohols.

No.	Z	X	α(diastereomer eluted faster)[a]		
			PYE	C_{18}	C_8
1	SO_2Ph	H[b]	1.26 (t)	1	1
2		$SiMe_3$[c]	1.21 (t)	1.05 (t)	1
3		COPh[c]	1.09 (t)	1.19 (t)	1.16 (t)
4	CONHPh	H[b]	1.08 (e)	1.10 (e)	1.08 (e)
5		$SiMe_3$[c]	1	1.07 (t)	1.07 (t)
6		COPh[c]	1.09 (t)	1.14 (t)	1.13 (t)

a); A letter in a bracket is a symbol to the diastereomer eluted faster, e:erythro, t:threo
b); Mobile phase: 80 % aqueous methanol, c); Mobile phase: 90 % aqueous methanol.

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bulkiness to afford anti conformation as depicted in Figure 8. The separations on the three stationary phases are summarized in Table 4.

By functionalization, on C_{18} and C_8 stationary phases, separation tends to be better than that of the alcohol, while on PYE stationary phase, the separation tents to be worse than that of the alcohol. Since the intramolecular hydrogen bonding is destroyed by these functinalizations to afford the anti conformation which may affect the balance of the hydrophobicity of the diastereomers, the elution order of the diastereomers including the amide functional group (Z) are reversed on C_{18} and C_8 stationary phases as expected. This finding is mainly because aliphatic C_{18} and C_8 stationary phases make molecular recognition based on the difference in the hydrophobicity of solutes. On the other hand, PYE stationary phase can recognize molecular planarity as well as the difference in the balance of the hydrophobicity of the solutes, so that, the elution orders are inconsistent each other because the difference in the molecular planarity becomes smaller by these functionalizations, but these functinalizations tend to make the separation worse.

In an aqueous acetonitrile mobile phase, the separation of the esterified diastereomer including phenylsulfonyl group (No. 2) as illustrated in Figure 8 was changed on PYE stationary phase to afford almost no separation, however, on C_{18} stationary phase, no

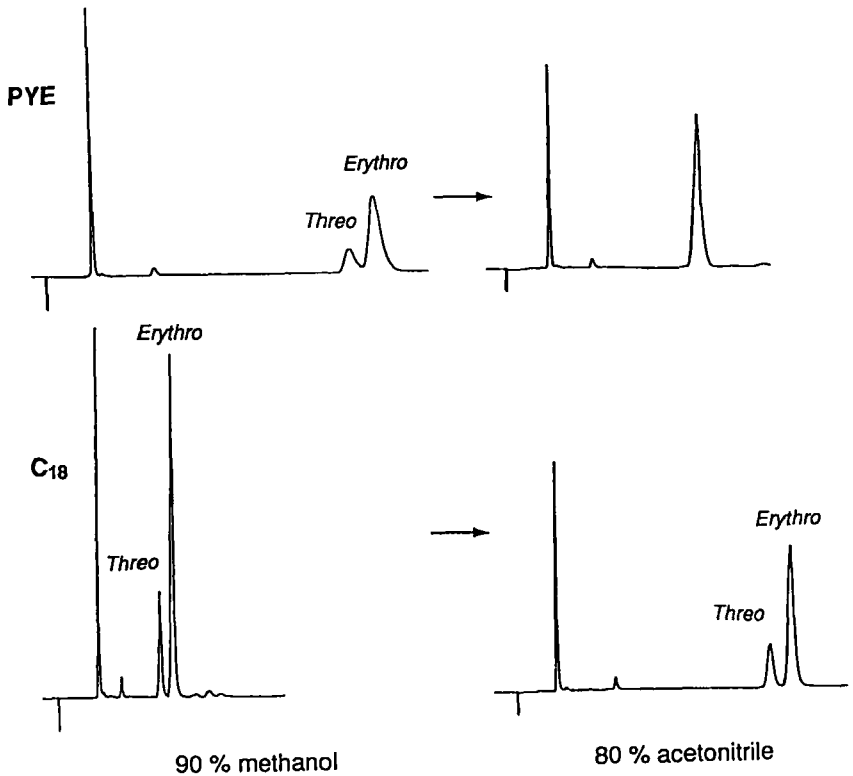

FIGURE 9. An effect of the mobile phase on the separation of the protected alcohols
Sample; No. 2 in Table 4.
Flow rate; 1 ml/min.
Detection; UV 254 nm

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significant change was found (Figure 9). These findings are very similar to the results found in a previous section and assist our prediction.

CONCLUSION

PYE stationary phase can separate the acyclic stereoisomers better than C_{18} and C_8 stationary phases in most cases when the same hydrophilic substituent is included. The

elution order on PYE phase can be explained based on the structural planarity of solutes and preferable retention for a functional group rather than the hydrophobic interaction, therefore when the preferable retention is lost by the change of the hydrophilic substituent, elution order tend to be changed, while there is almost no change in the elution order by the change of the hydrophobic alkyl substituents. On the other hand, aliphatic C_{18} and C_8 stationary phases separate solutes based on the difference in the hydrophobicity. So, on C_{18} and C_8 stationary phases, the change of the hydrophobic alkyl substituents tends to make a drastic changes in the elution orders, however, the change of the hydrophilic functional group does not make any significant change in the elution orders of diastereomers. The functionalization of the hydroxy group is effective to separate the diastereomers on C_{18} and C_8 stationary phases when the balance of the hydrophobicity is quite changed, while this is not so effective for the separation on PYE stationary phase.

In conclusion, PYE stationary phase and C_{18} stationary phase are useful for a molecular recognition for acyclic stereoisomer even in a reversed phase mode if an appropriate combination is made.

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