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EFFECT OF INTERSTRAND DISTANCE UPON CHIRAL RECOGNITION BY A CHIRAL STATIONARY PHASE

WILLIAM H. PIRKLE* and MYUNG HO HYUN
School of Chemical Sciences, University of Illinois, Urbana, IL 61801 (U.S.A.)
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SUMMARY

For a silica-bonded α-arylalkylamine-derived chiral stationary phase, interstrand distance has a notable effect upon the relative contributions of two competing chiral recognition processes. As the strands become more densely packed, intercalative processes become more difficult. By default, the contribution from non-intercalative processes is increased. For analytes which show preference for the intercalative process, a chiral stationary phase having widely spaced strands affords the greatest enantioselectivity. Closely spaced strands are best for those analytes which preferentially utilize the non-intercalative process.

INTRODUCTION

The chromatographic separation of enantiomers is assuming great importance in most branches of the medicinally or chemically oriented sciences. This technique has developed rapidly over the past few years owing, in large measure, to the snowballing effect of insight into the details of chiral recognition mechanisms.

Despite the obvious effect this insight has had on the design of chiral stationary phases, there are still reservations in some quarters as to whether any chiral recognition mechanism yet postulated actually bears much semblance to reality. The point is made that, since enantiomers undergo a myriad of transitory interactions as they pass over the chiral stationary phase, it is simplistic to think that the difference in their weighted time-average behavior can be explained by relatively simple models. The countering argument is that most of these transitory interactions do not lead to enantioselectivity and therefore can be largely neglected. Basically, one is only concerned with those few retention mechanisms which make significant contributions to enantioselectivity. Although chiral recognition mechanisms are usually inferred from a body of chromatographic data and cannot be rigorously proven, we consider this approach to be capable of producing good first approximations of the actual mechanisms by which enantioselection is achieved. Undoubtedly, our ability to describe these mechanisms will progress as data accumulate. Even so, the present mechanisms do rationalize a vast amount of data and are of obvious predictive power. Because interpretation of the data is complex and the postulated mechanisms are, in the minds

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of some, of uncertain validity, we feel it to be important to test these models as completely as possible by whatever techniques can be brought to bear.

EXPERIMENTAL

Chromatography

Chiral stationary phases were packed into 250 × 4.6 mm I.D. stainless-steel columns as a methanol slurry. Chromatography was performed using an Altex 100A pump, an Altex 210 injector, an Altex 165 variable-wavelength UV detector and a Kipp en Zonen BD 41 recorder. All analytes used in this study were prepared by the methods reported previously[1].

Chiral stationary phase 1 with different loading

Medium loaded chiral stationary phase 1 has been reported elsewhere[1,2]. Heavily and lightly loaded chiral stationary phase 1 were prepared by a slightly modified method as follows.

To the solution of (*R*)-N-(10-undecenoyl)-α-(6,7-dimethyl-1-naphthyl)-isobutylamine[1] (1 g for highly loaded chiral stationary phase 1, 250 mg for lightly loaded chiral stationary phase 1) in trichlorosilane (15 ml) was added 1 ml of an isopropanol solution of chloroplatinic acid (30 mg in 20 ml isopropanol) with stirring under a nitrogen atmosphere. After heating to reflux for 20 min, the excess trichlorosilane was removed by simple distillation under a nitrogen atmosphere. Residual trichlorosilane was evaporated under high vacuum. The residue was dissolved in benzene (20 ml) and added to 5 g of 5-μm Spherisorb silica gel which has been dried by azeotropic distillation of water (Dean–Stark trap) from a slurry in 50 ml of benzene. This silica gel slurry (*ca.* 70 ml) was heated to reflux for 3 h under a nitrogen atmosphere. The cooled silica gel slurry was filtered and the silica was washed with benzene, ethyl acetate, methanol, acetone, diethyl ether, and pentane.

Heavily loaded chiral stationary phase 1: Anal., found: C, 11.44; H, 1.59; N, 0.67; Si, 39.85; Cl, trace. calcd.: 0.48 mmoles/g (based on N); 0.35 mmoles/g (based on C).

Lightly loaded chiral stationary phase 1: Anal., found: C, 3.79; H, 0.65; N, 0.19; Si, 44.30; Cl, trace. calcd.: 0.14 mmoles/g (based on N): 0.10 mmoles/g (based on C).

Filled chiral stationary phase 1

Moderately loaded chiral stationary phase 1 (15 g) was prepared by the procedure described above. This chiral stationary phase was divided into three portions. One portion was packed into a stainless-steel column. The other two portions were treated with either *n*-octyldimethylchlorosilane or *n*-butyltrichlorosilane, as follows.

One portion (5 g) of chiral stationary phase 1 was slurried with benzene and refluxed to remove water azeotropically using a Dean–Stark trap. After removing water completely, a solution of *n*-octyldimethylsilane (2 ml) or *n*-butyltrichlorosilane (2 ml) in benzene (20 ml) was added to the chiral stationary phase slurry. After heating the slurry to reflux for 3 h under a nitrogen atmosphere, the filled chiral stationary phase 1 was filtered and washed with benzene, ethyl acetate, methanol, acetone, diethyl ether, and pentane.

Original moderately loaded chiral stationary phase 1: Anal., found: C, 5.35; H, 0.83; N, 0.30; Si, 42.92; Cl, trace. Calcd.: 0.21 mmoles/g (based on N); 0.16 mmoles/g (based on C).

n-Octyl-filled chiral stationary phase 1: Anal., found: C, 6.75; H, 1.15; N, 0.19; Si, 42.34; Cl, trace.

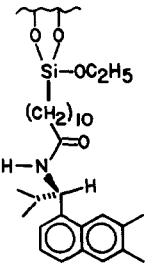
n-Butyl-filled chiral stationary phase 1: Anal., found: C, 7.12; H, 1.17; N, 0.19; Si, 41.64; Cl, trace.

RESULTS AND DISCUSSION

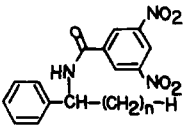
We recently described a series of chiral stationary phases on which one can separate the enantiomers of a host of amines, amino alcohols and amino acids, all as their N-3,5-dinitrobenzoyl derivatives[1–3]. These phases were suggested to utilize two competing chiral recognition processes of opposite enantioselectivities[2,3]. The relative extent to which each process contributes to the overall separation was suggested to be a function of stationary phase structure, analyte structure, and mobile phase composition. We now show that the distance between the strands of bonded phase also influences the contribution of each process and, moreover, does so in a way that supports the postulated mechanisms.

Columns containing chiral stationary phase 1 bonded to 5-μm Spherisorb silica gel at three different loading levels were prepared either as previously described or by the variation described herein. A homologous series of α-phenylalkylamine derivatives, 2, were chromatographed using 2-propanol–hexane (20:80) as the mobile phase. Fig. 1 shows the relation between separability factor, length of the analyte's alkyl "tail", and the degree to which each of the three columns is loaded with strands of the chiral amide. Changes in 2-propanol concentration alter retention but have little effect on selectivity.

Rather than recapitulate the details of the prior chiral recognition arguments[2,3], we will simply reassert that type 2 analytes are retained on (*R*)-chiral sta-



1



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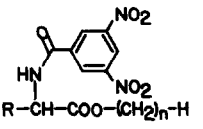

3a R= C_6H_5
b R= $C_6H_5CH_2$

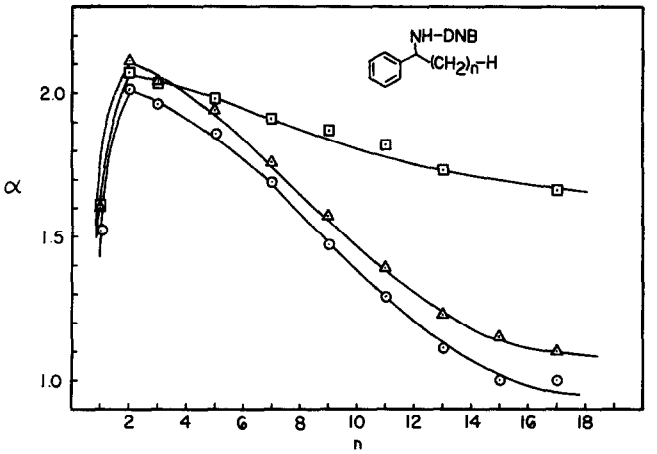

Fig. 1. Resolutions of type 2 analytes on columns containing light (⊡), medium (△), or heavy (⊙) loadings chiral stationary phase 1.

tionary phase 1 by an intercalative dipole-stacking process which selectively retains the (*R*)-enantiomers and by a relatively non-intercalative hydrogen-bonding process which selectively retains the (*S*)-enantiomers. These are depicted rather simplistically in Fig. 2. On the basis of this model, one expects that a "closing of the ranks" among the strands of bonded phase would make the intercalative process more difficult and thus, by default, allow greater contribution from the non-intercalative process. Moreover, this effect should be greatest for those analytes having to intercalate the longest alkyl tails. From Fig. 2, we see that when the alkyl tails are short, the degree of loading, low (*ca.* 0.1 mmole/g), medium (*ca.* 0.2 mmoles/g), or high (*ca.* 0.4 mmoles/g) has relatively little effect on the magnitudes of the enantiomer separability factors, α^*. However, as the tails lengthen, the initially dominant intercalative process plays a diminishing role and this diminishment is greatest on the heavily loaded column. This is the trend expected from the mechanistic hypothesis.

Esters of N-(3,5-dinitrobenzoyl)-amino acids are also subject to competing chiral recognition processes of opposite enantioselectivity[2,3]. For these analytes, the non-intercalative process is initially dominant, presumably because of the added hydrogen-bonding site, the carboalkoxy carbonyl oxygen. Hence, the (*R*)-enantiomers (remember the inversion in substituent priority) are selectively retained. Chromatography of a homologous series of esters, 3ab, of N-3,5-dinitrobenzoylphenylglycine and N-3,5-dinitrobenzoylphenylalanine on the three type 1 columns results in the data depicted in Fig. 3 (for the phenylglycine series) and in Table I.

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* The maximization of α at $n = 2$ is a property of type 2 analytes and deserves comment. This maximization is presumed to arise as a consequence of the effect the size of the alkyl substituent has upon the conformational behavior of the analyte. Ethyl is significantly larger than methyl and exerts a greater degree of conformational control, more heavily populating conformations favorable for chiral recognition. However, alkyl groups longer than ethyl are not effectively larger near the chiral center and hence provide little additional conformational control. However, longer alkyl groups do begin to interfere with the dipole-stacking process.

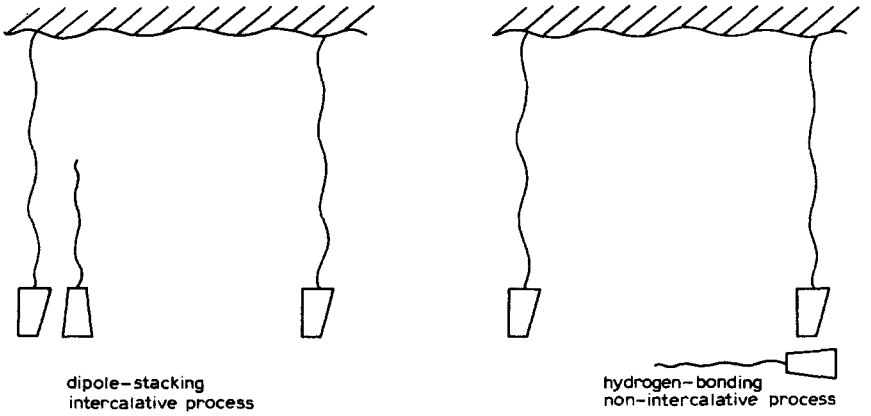

Fig. 2. Schematic presentation of the two competing chiral recognition processes.

One expects that lengthened alkoxy tails will reduce the contribution of the intercalative process and further enhance the already dominant non-intercalative process. Again, this effect is expected to be greatest for the column containing the silica most heavily loaded with the chiral amide and for the analyte having the longest alkoxy tails. This is precisely the observed result. Thus, the type 3 analytes again support the occurrence of competing intercalative and non-intercalative processes of opposite enantioselectivities.

Interestingly, whether the lightly or the heavily loaded column affords the greates α values depends upon whether the intercalative or non-intercalative process is dominant. This trend can be used to ascertain the mechanistic preference of a series of analytes, a helpful bit of information if one is trying to relate elution order to absolute configuration. In most instances, the more heavily the silica is loaded with

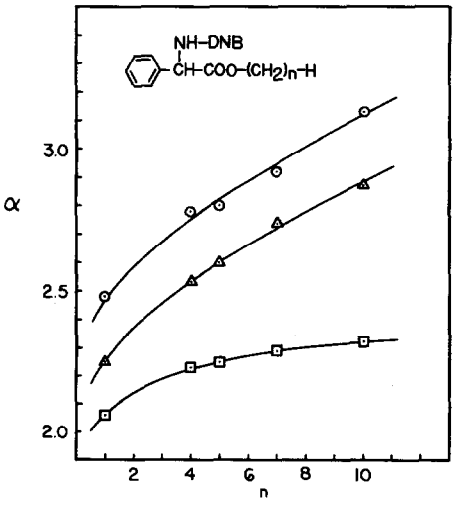

Fig. 3. Resolution of type 3a analytes on columns containing light (⊡), medium (△), or heavy (⊙) loadings of chiral stationary phase 1.

TABLE I

RESOLUTION OF ENANTIOMERS ON CHIRAL STATIONARY PHASE 1 AT DIFFERENT LOADING LEVELS

Compound	*n*	*Lightly loaded*			*Medium loaded*			*Heavily loaded*		
		α*	k'_1**	*Conf.***	α*	k'_1**	*Conf.***	α*	k'_1**	*Conf.***
2	1	1.61	8.8	*R*	1.60	11.1	*R*	1.52	16.7	*R*
	2	2.07	9.7		2.11	16.3		2.01	17.4	
	3	2.03	10.8		2.04	15.8		1.96	15.4	
	5	1.98	10.2		1.94	17.7		1.86	14.5	
	7	1.92	9.5		1.76	15.9		1.69	13.6	
	9	1.87	8.5		1.57	14.3		1.47	12.3	
	11	1.82	7.8		1.39	13.4		1.29	11.4	
	13	1.73	8.2		1.23	12.4		1.11	10.6	
	17	1.66	6.2		1.10	10.9		1.00	9.6	
3a	1	2.06	6.5	*R*	2.25	11.6	*R*	2.48	11.9	*R*
	4	2.23	4.6	*R*	2.53	7.1	*R*	2.78	7.3	*R*
	5	2.25	4.4	*R*	2.60	6.4	*R*	2.80	6.9	*R*
	7	2.29	3.8	*R*	2.74	5.3	*R*	2.92	5.6	*R*
	10	2.32	3.2	*R*	2.87	4.3	*R*	3.13	4.3	*R*
3b	1	3.43	5.5	*R*	4.50	8.7	*R*	4.99	7.9	*R*
	4	3.45	4.0	*R*	4.71	5.4	*R*	5.06	4.9	*R*
	7	3.51	3.3	*R*	5.15	4.0	*R*	5.59	3.5	*R*
	10	3.38	2.8	*R*	5.64	3.2	*R*	6.06	2.8	*R*

* Chromatographic separability factor.
** Capacity factor for the first eluted enantiomer. Mobile phase is 2-propanol–hexane (20:80).
*** Absolute configuration of the second eluted enantiomer.

the chiral amide, the greater the capacity ratios of the enantiomers. However, this generalization breaks down for the heavily loaded column when type 2 analytes having long tails are chromatographed. One might suppose this is a consequence of extensive suppression of the intercalative retention process. The intercalative process is the dominant process for type 2 analytes in terms of contribution to chiral recognition. While this does not require that the intercalative process also be dominant in terms of its contribution to retention, this would be a reasonable inference from the data. In other words, the strongest interactions give rise to the greatest chiral recognition. We hasten to add that mechanistic arguments based on retention are less certain than those based on differential retention (*i.e.* separability factors).

One might ask whether closely spaced strands interact with one another and what effect this might have upon chiral recognition. To whatever extent this may occur, one expects reduced retention and reduced chiral recognition*. Since the heavily loaded column affords the greatest retention and the greatest enantioselectivity

* Similar effects have been noted previously in systems where closely spaced silica-bound ligands are "bridged" by complexing metal ions[4]. The present chiral stationary phase is expected to show a much reduced tendency for interstrand interaction.

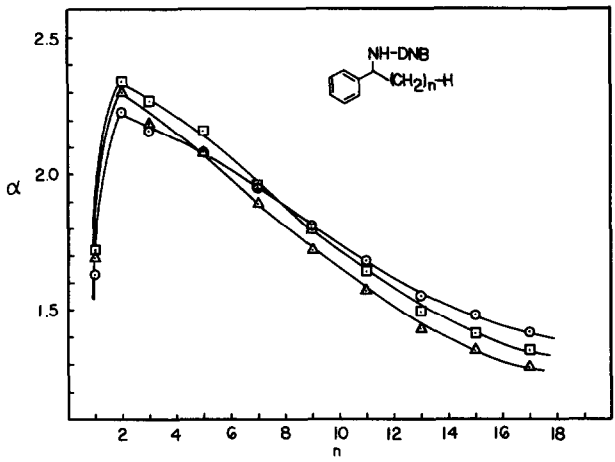

Fig. 4. Resolution of type 2 analytes on columns containing chiral stationary phase 1 (⊙), *n*-octyl-filled chiral stationary phase 1 (⊡), or *n*-butyl-filled chiral stationary phase 1 (△).

for the amino acid derivatives, interstrand interactions seem not to be of much significance in the present chiral stationary phases.

On the basis of the preceding arguments, one should be able to anticipate the effects of filling the space between the strands of the bonded chiral phase with inert alkyl strands. The inert alkyl strands should render the intercalative process relatively less favorable and reduce the ability of the stationary phase to retain either enantiomer.

Filled chiral stationary phases were prepared by treating portions of a different batch of moderately loaded (*ca.* 0.14 mmoles/g) chiral stationary phase 1 with *n*-octyldimethylchlorosilane or *n*-butyltrichlorosilane. The added loadings were *ca.* 0.03

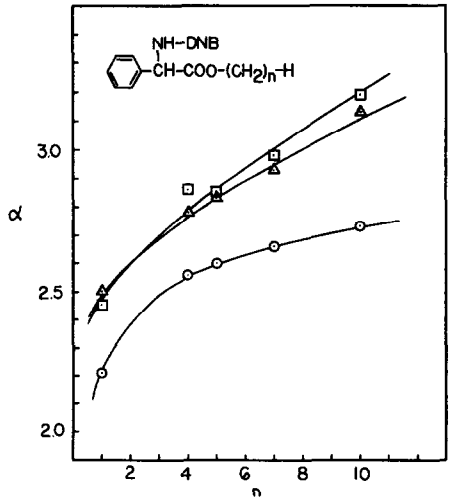

Fig. 5. Resolution of type 3a analytes on columns containing chiral stationary phase 1 (⊙), *n*-octyl-filled chiral stationary phase 1 (⊡), or *n*-butyl-filled chiral stationary phase 1 (△).

TABLE II

RESOLUTION OF ENANTIOMERS ON CHIRAL STATIONARY PHASE 1 MODIFIED BY "FILLING" WITH *n*-OCTYL OR *n*-BUTYL GROUPS

Compound	*n*	*Unfilled**			*n-Octyl-filled*			*n-Butyl-filled*		
		α**	k'_1***	*Conf.*§	α**	k'_1***	*Conf.*§	α**	k'_1***	*Conf.*§
2	1	1.63	11.8	*R*	1.72	10.2	*R*	1.69	10.2	*R*
	2	2.23	12.9		2.34	11.4		2.30	11.4	
	3	2.16	13.6		2.27	12.1		2.19	12.3	
	5	2.08	13.0		2.16	11.8		2.08	12.0	
	7	1.95	11.6		1.96	10.7		1.89	10.9	
	9	1.81	10.6		1.80	9.7		1.72	10.0	
	11	1.68	9.8		1.64	9.2		1.57	9.2	
	13	1.55	9.3		1.49	8.5		1.43	8.6	
	15	1.48	8.6		1.41	8.1		1.35	8.1	
	17	1.42	8.2		1.35	7.8		1.29	7.9	
3a	1	2.21	8.3	*R*	2.45	6.0	*R*	2.50	6.4	*R*
	4	2.45	5.3	*R*	2.86	4.0	*R*	2.78	4.5	*R*
	5	2.60	4.9	*R*	2.85	3.9	*R*	2.83	4.1	*R*
	7	2.66	4.1	*R*	2.98	3.3	*R*	2.93	3.5	*R*
	10	2.73	3.5	*R*	3.2	2.6	*R*	3.13	2.8	*R*
3b	1	4.28	6.4	*R*	4.89	4.5	*R*	4.71	4.9	*R*
	4	4.50	4.1	*R*	4.98	3.3	*R*	5.07	3.3	*R*
	7	4.94	3.1	*R*	5.68	2.4	*R*	5.50	2.6	*R*
	10	5.28	2.5	*R*	6.21	2.0	*R*	5.58	2.2	*R*

* See Experimental section for loading.
** Chromatographic separability factor.
*** Capacity factor for the first eluted enantiomer. Mobile phase was 2-propanol–hexane (20:80).
§ Absolute configuration of the second eluted enantiomer.

mmoles/g and 0.06 mmoles/g, respectively*. Columns were prepared from the control portion of moderately loaded 1 (*i.e.* 0.14 mmoles/g), the octyl-filled 1 and the butyl-filled 1. Figs. 4 and 5 show the results of chromatographing the series 2 and series 3 analytes on these columns. Table II presents the relevant chromatographic data.

As expected, both filled chiral stationary phases give smaller α values for long-tailed type 2 analytes and larger α values for the amino acid derivatives than does the unfilled parent chiral stationary phase . Filling reduces k'_1 values, as expected. Although the larger *n*-octyldimethylsilane was introduced onto chiral stationary phase 1 to a lesser extent than the smaller and more reactive *n*-butyltrichlorosilane, the greater length of the former seems to compensate for the lower loading level.

In the case of short-tailed type 2 analytes, the filled chiral stationary phases afford somewhat greater enantioselectivity as well as reduced retention. The greater enantioselectivity was not expected from mechanistic considerations and may stem

* These values are based on elemental analysis and involve some assumptions as to the number and nature of alkoxy groups bonded to the silicon atoms of the various organosilanes. The values given should be considered as approximate values only.

simply from an end-capping of residual silanol groups that afford retention without enantioselectivity. However, once the alkyl tails become long enough to interfere with the filling moieties, the reduction in the contribution of the dipole-stacking process causes the filled chiral stationary phases to be inferior to the unfilled chiral stationary phase.

The preceding discussion has implicitly used the terms intercalative and non-intercalative as applying to the alkyl or alkoxy tails of the type 2 or 3 analytes. We have neither explicitly considered the intercalation of nor the effect of structural variation within the remaining chiral-center substituent (*i.e.*, the phenyl or benzyl group). The effect such structural variation has upon the balance between the competing chiral recognition processes is being studied.

ACKNOWLEDGEMENTS

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