Journal of Chromatography, 348 (1985) 89–96
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 079

CHROMATOGRAPHIC SEPARATION OF THE ENANTIOMERS OF 2-CARBOALKOXYINDOLINES AND N-ARYL-α-AMINO ESTERS ON CHIRAL STATIONARY PHASES DERIVED FROM N-(3,5-DINITROBENZOYL)-α-AMINO ACIDS

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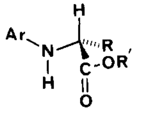
(Received July 30th, 1985)

SUMMARY

N-Aryl-α-amino esters, 1, may be considered to be acyclic analogues of 2-carboalkoxyindolines, 2. The enantiomers of both types of compounds may be separated chromatographically on chiral stationary phases (CSPs) prepared from N-(3,5 dinitrobenzoyl) derivatives of (*S*)-leucine and (*R*)-phenylglycine. The degree of chiral recognition is high. Separation factors greater than 10 have been noted and preparative separations are readily performed. A chiral recognition model is advanced to account for the observed separations and is used to relate elution order to absolute configuration for a series of type 1 and type 2 analytes.

INTRODUCTION

From a partial understanding of chiral recognition requirements, it was anticipated that one might chromatographically separate enantiomers of N-aryl-α-amino esters, 1, and 2-carboalkoxyindolines, 2, on chiral stationary phases (CSPs) 3 and 4. These CSPs, derived respectively from the 3,5-dinitrobenzamides of (*R*)-phenylglycine and (*S*)-leucine, have proven capable of separating a broad spectrum of racemates on both analytical and preparative scales[1]. Compounds of general type 1 and 2 contain appropriate sites (π-donor, hydrogen bond donor and hydrogen bond acceptor sites) at which they can undergo the multiple simultaneous interactions essential for chiral recognition on CSPs 3 and 4. Prior experience has shown that



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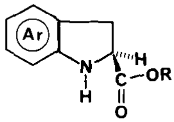

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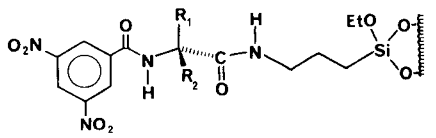


3 R_1 = Phenyl, R_2 = H
4 R_1 = H, R_2 = isobutyl

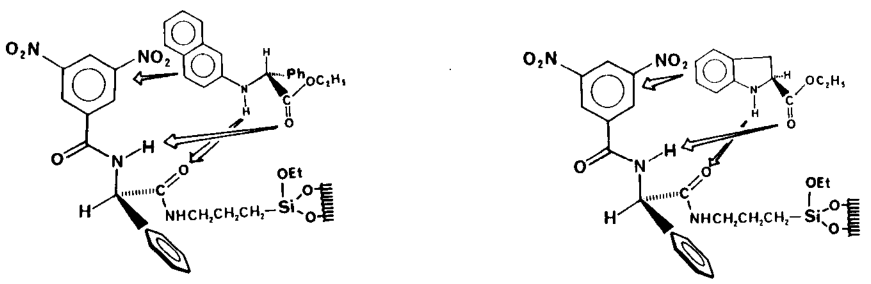
enantiomers containing this combination of complimentary sites can be resolved on these CSPs and that the enantiomer having the clockwise arrangement: π donor site → basic site → acidic site on the molecular "face" presented to CSP (*R*)-3 will be most strongly retained[2,3]. This is illustrated in Fig. 1.

The preceeding expectations were fulfilled, and this paper describes a general approach to the resolution of compounds of type 1 and 2. Since the indoline moiety is ubiquitous in biological molecules, the present approach could have far reaching utility as a means of obtaining enantiomerically pure, configurationally known indoline derivatives.

Esters of N-aryl-α-amino acids show very large separation factors when chromatographed on CSPs 3 and 4. This high selectivity indicates that chiral recognition is quite efficient and results from rather specific interactions between the CSP and the analytes. Thus, the study of these compounds should allow further clarification of the nature of chiral recognition. Moreover, these compounds themselves are attractive candidates for incorporation into reciprocal CSPs intended for use in the resolution of the 3,5-dinitrobenzamides of chiral amino acids, amines, etc.[4].

EXPERIMENTAL

The columns used in this study are commercial versions (J. T. Baker) of the types described earlier[1]. The high performance liquid chromatography system used in this study consists of a Beckman 210 injector, a Beckman Model 100A pump, a Rudolph Autopol III recording polarimeter equipped with a 20-cm flow cell, and a Beckman 165 variable wavelength detector equipped with an Altex Model C-R1A recording integrator.



1c 2a

Fig. 1. Interaction between CSP (*R*)-3 and the most retained enantiomers of 2a and 1c.

All samples were characterized by NMR spectroscopy and elemental analysis. Preparation of a number of these compounds is being reported elsewhere. The N-aryl analogues of phenylglycine, 1a–d and 1n–s, were prepared by the method of Baker[5]. All other type 1 compounds were prepared as described earlier[6].

The 2-carboalkoxyindolines, 2a–e, were prepared by tin reduction of the appropriate 2-carboalkoxyindoles. These indoles were prepared by a variation of the Reissert synthesis or by the method of Hemetsberger[7]. It is important to note that indolines of this type will re-aromatize in the presence of oxygen and should be handled and stored under an inert atmosphere.

RESULTS AND DISCUSSION

Typical separations of the enantiomers of several 2-carboalkoxyindolines are shown in Table I. Table II gives a similar listing for a series of N-aryl-α-amino esters. Fig. 2 depicts a typical chromatogram and illustrates the chromatographic parameters: α, the separability factor, and *k'*, the capacity ratio.

TABLE I

SEPARATION OF ENANTIOMERS OF 2-CARBOALKOXYINDOLINES, 2, ON CSP (*R*)-3 WITH 10% 2-PROPANOL IN HEXANE AS MOBILE PHASE

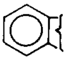
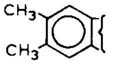
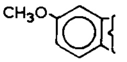
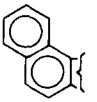
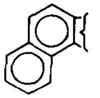
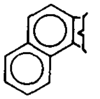
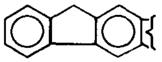
Compound	*Ar*	*R*	α	*Most retained enantiomer*
2a		Ethyl	1.50	*S*-(+)
2b		Ethyl	1.28	
2c		Ethyl	1.63	
2d		Ethyl	2.20	(+)
2e		Ethyl	2.90	(+)
2f		Isopropyl	2.60	(+)
2g		Ethyl	1.85	(+)

TABLE II

SEPARATION OF ENANTIOMERS OF N-ARYL α-AMINO ESTERS 1

Compound	*Ar*	*R*	*R′*	*α*	*k′*	*CSP*	*Mobile phase (% 2-propanol in hexane)*	*Most retained enantiomer*
1a	Phenyl	Phenyl	Ethyl	1.25	1.68	3	2	(−)
1b	1-Naphthyl	Phenyl	Ethyl	1.35	1.62	3	2	(−)
1c	2-Naphthyl	Phenyl	Ethyl	2.15	2.09	3	2	*R*-(−)
1d	2-Anthryl	Phenyl	Ethyl	2.50	5.82	3	2	(−)
1e	2-Anthryl	Methyl	Ethyl	8.28	11.98	4	1	(−)
1f	2-Naphthyl	Methyl	Ethyl	9.80	11.10	4	1	*S*-(−)
1g	2-Naphthyl	Ethyl	Ethyl	8.70	11.60	4	1	(−)
1h	2-Naphthyl	*n*-Propyl	Ethyl	9.04	13.40	4	1	(−)
1i	2-Naphthyl	Isopropyl	Ethyl	8.00	10.24	4	1	*S*-(−)
1j	2-Naphthyl	*n*-Butyl	Ethyl	8.61	10.35	4	1	(−)
1k	2-Naphthyl	Isobutyl	Ethyl	8.08	9.77	4	1	*S*-(−)
1l	2-Naphthyl	Cyclohexyl	Ethyl	6.09	5.78	4	1	(−)
1m	2-Naphthyl	Methyl	Undecenyl	10.51	5.94	4	1	*S*-(−)
1n	2-Naphthyl	Phenyl	Methyl	1.87	7.60	3	2	*R*-(−)
1o	2-Naphthyl	Phenyl	Ethyl	2.33	5.46	3	2	*R*-(−)
1p	2-Naphthyl	Phenyl	Isopropyl	2.50	5.08	3	2	*R*-(−)
1q	2-Naphthyl	Phenyl	*n*-Butyl	2.47	4.90	3	2	*R*-(−)
1r	2-Naphthyl	Phenyl	*n*-Heptyl	2.50	4.57	3	2	*R*-(−)
1s	2-Naphthyl	Phenyl	*n*-Decyl	2.58	4.41	3	2	*R*-(−)
1t	2-Naphthyl	Methyl	Ethyl	6.50	4.26	4	5	*S*-(−)
1u	2-Naphthyl	Ethyl	Ethyl	5.77	3.54	4	5	(−)
1v	2-Naphthyl	*n*-Propyl	Ethyl	4.85	3.82	4	5	(−)
1w	2-Naphthyl	*n*-Butyl	Ethyl	4.28	1.66	4	5	(−)
1x	2-Naphthyl	*n*-Pentyl	Ethyl	3.96	1.70	4	5	(−)
1y	2-Naphthyl	*n*-Hexyl	Ethyl	3.84	1.53	4	5	(−)
1z	2-Naphthyl	*n*-Heptyl	Ethyl	3.72	1.48	4	5	(−)
1aa	2-Naphthyl	Methyl	*n*-Decyl	8.83	7.99	4	1	(−)
1bb	N-(1-Naphthyl)α-amino-γ-butyrolactone			1.17	15.50	4	5	(+)
1cc	N-(2-Naphthyl)α-amino-γ-butyrolactone			1.22	21.25	4	5	(+)
1dd	N-(p-Methylphenyl)α-amino-γ-butyrolactone			1.18	8.00	4	5	(+)

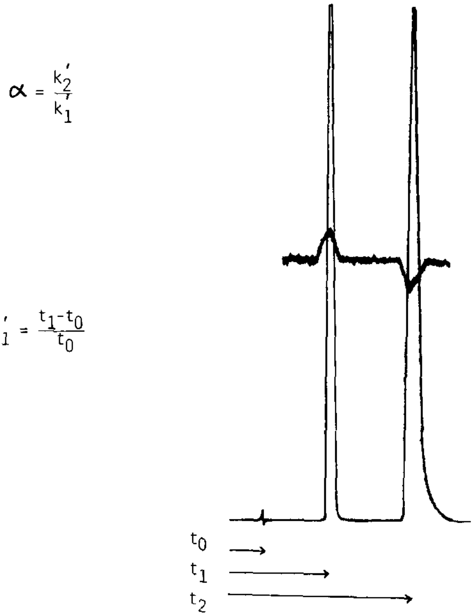


Fig. 2. Separation of the enantiomers of 1c on CSP (*R*)-3. The lower trace is absorption at 254 nm, the upper trace is polarimeter response at 589 nm.

The racemates in these studies were prepared using relatively simple procedures. N-aryl amino esters, 1, were prepared by the alkylation of primary aromatic amines with α-halo esters[5,6]. The 2-carboalkoxyindolines, 2, were prepared by reduction of the corresponding 2-carboalkoxyindoles[8] which were in turn synthesized from the appropriate aromatic aldehydes and the sodium enolate of ethyl 1-azidoacetate[7,9].

The absolute configuration of 2-carboethoxyindoline, 2a, was established by reduction of one chromatographically obtained enantiomer with lithium aluminium hydride to the configurationally known 2-hydroxymethylindoline[8]. Elution orders of the enantiomers of N-aryl-α-amino esters were determined by chromatography of enantiomerically enriched samples prepared by arylation of the appropriate α-amino ester with the bisulfite addition product of β-naphthol —a variation of the classical Bucherer reaction.

Depicted in the drawings of 1 and 2 are the conformations which are expected to be most stable in solution. The 2-carboalkoxyindolines are relatively rigid; only the carboalkoxy group can rotate. For steric reasons, the N-aryl-α-amino esters will preferentially populate the rotamer that places the methine hydrogen near the plane of the aryl substituent. The larger the R substituent, the greater the tendency to populate this rotamer. In both compound types, the nitrogen can be taken as essentially planar owing to delocalization of the nitrogen lone pair into the aryl substituent. Hydrogen bonding between the carbonyl oxygen and the amine hydrogen can, for both types of compounds, influence the conformational behavior of the carboalkoxy substituent. The occurence of intramolecular hydrogen bonding is inferred from the downfield positions of the NH resonances in the ^{1}H NMR spectra of these compounds at high dilution, and from their non-polar behavior in terms of solubility and chromatography.

From the drawings of 1 and 2, one notes, when viewing the face presenting the three complimentary sites, that there is a similar clockwise arrangement of the three sites (π donor, hydrogen bond donor and hydrogen bond acceptor) for the (*R*)-enantiomer of 1 and the (*S*)-enantiomer of 2. Experimentally, one notes that it is the *S*-(+) enantiomer of 2a and the *R*-(−) enantiomer of 1c which elute last from CSP (*R*)-3. The elution orders are reversed on (*S*)-4, as expected.

Our initial feeling was that the aryl portions of type 1 and 2 analytes would be utilized as π-donor sites, forming van der Waals ("π–π") complexes with the 3,5-dinitrobenzoyl moiety of the CSP. Note that the magnitude of α. the separability factor, increases (as does the capacity ratio, k') as the π-donor nature of the aryl unit is increased (1a–d, 2a < 2e < 2c). This stems from a strengthening of the van der Waals π–π interaction involved in the chiral recognition process.

We expected the carbonyl oxygen of the analytes to be used as a basic site for hydrogen bonding by the 3,5-dinitrobenzoyl-N*H*. In turn, the analyte's aniline-like N*H* was expected to hydrogen bond to the C-terminal carboxamide carbonyl of the CSP. Replacement of either the carboalkoxy group or the aniline-like N*H* with a methyl group (*e.g.*, 2-methyl indoline or ethyl N-methyl-N-phenyl phenyglycinate) alters the chromatographic behavior of the racemate and enantiomer separation is no longer observed. Fig. 1 illustrates the expected simultaneous occurrence of the aforementioned interactions between CSP (*R*)-3 and the most retained enantiomers: (*S*)-(+)-2a and (*R*)-(−)-1c.

The two drawings represent variations on the same chiral recognition model. The analyte enantiomers which undergo the depicted interactions will be most strongly retained. By interchanging two groups on the chiral centers of the analytes depicted in Fig. 1, it can be seen that the other (less-retained) enantiomer of the analytes can, at best, undergo only two of the three required interactions at any one time, and will thus form a less stable diastereomeric adsorbate. Since the absolute configuration of CSP 4 is opposite that of CSP 3, elution orders are reversed. That is, the most retained enantiomer on CSP 3 should be the least retained on CSP 4. Inspection of Table III will show that this is the case. Among compounds for which absolute configurations are known, we find no instance in which the elution order of type 1 or 2 analytes does not conform to that expected on the basis of the models in Fig. 1. In cases where the absolute configuration has not been otherwise established, the correlation between the sign of rotation and elution order is uniform* and matches that of analytes of known configuration. On this basis, we assign the (*R*)-1 and (*S*)-2 configurations to the analyte enantiomers listed in Tables I and II which are most strongly retained on CSP (*R*)-3.

It has already been noted that type 2 analytes are conformationally quite rigid, while type 1 analytes retain a degree of flexibility. We believe this flexibility accounts, at least in part, for the greater degree of separation seen for the type 1 analytes. It has been commonly assumed that steric rigidity increases chiral recognition or en-

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* It is to be noted that the presence of the phenyl substituent on the chiral center causes all the phenylglycine derivatives to differ in sign of rotation from the remaining alkyl-substituted type 1 analytes.

TABLE III

SEPARATION OF ENANTIOMERS OF 1m ON CSP (*S*)-4

The *S*-(−)-1m is last eluted.

Temperature (°C)	*Separability factors (α)*	
	Percentage of 2-propanol in hexane mobile phase	
	1	*5*
0	12.82	10.01
20	10.51	6.75
65	5.46	3.90

hances asymmetric induction during asymmetric synthesis. This is not necessarily so, and the type 1 and 2 analyte comparison illustrates this quite nicely. For efficient chiral recognition, the most retained enantiomer must conform closely to the lowest energy conformation of the CSP. Clearly, the cyclic type 2 analytes do not have the flexibility of the type 1 analytes and cannot as easily conform to the shape of the CSP. This can also be seen in the lower separability of 1bb–1dd, (derivatives of α-amino-γ-butyrolactone) with respect to other type 1 analytes. Significantly, the acyclic type 1 compounds show increased chiral recognition as the size of the alkyl substituent R decreases (1f–1l). This decrease in substituent bulk must permit more conformational freedom. This is most noticeable in the case of R = isopropyl (1i, α = 8.08) *versus* R = *n*-propyl (1h, α = 9.04) and R = isobutyl (1k, α = 8.00) *versus* R = *n*-butyl (1j, α = 8.61), indicating that steric bulk close to the chiral center is detrimental to chiral recognition. It is also notable that CSP 4 affords greater chiral recognition of type 1 analytes than does CSP 3, presumed on other grounds to be the more sterically rigid of the two CSPs.

On the other hand, the gradual reduction of α as linear alkyl R groups increase in length (1t–1z) is likely to be due to steric interaction between the alkyl group and either the underlying silica support or the neighboring strands of bonded phase. The chiral recognition model requires that this alkyl group be intercalated between the strands of bonded phase. This intercalation effect has been noted in other systems to influence the degree of chiral recognition[2,3].

The trend of increasing separability with increasing alkoxy chain length (1m–1s) is explained by noting that in the more stable complex (Fig. 1) the alkoxy group is directed away from the CSP and the underlying silica support and undergoes no strong interaction with either. However, the alkoxyl group must intercalate between the strands of the CSP in the case of the less retained enantiomer if the π–π and dinitrobenzamide N*H*-carbonyl oxygen interactions are to be maintained. This has the consequence of reducing the retention of the less retained enantiomer and increasing the magnitude of the separability factor.

Experience has shown that solvent polarity has a minimal effect upon the magnitude of the separability factor for enantiomers chromatographed on CSPs 3 and 4. Absolute retention values (k') are affected but not the ratios of these values. This generalization, however, does not hold well for type 1 analytes, as may be seen from the data on Table III. The origin of this dependence of separability factor magnitude upon solvent polarity is not known with certainty, but may stem from competition between 2-propanol and the aniline-like N*H* for hydrogen bonding at the basic site of the CSP. In the adsorbate, this hydrogen bond may be considered to be intramolecular. Intermolecular hydrogen bonds usually do not compete effectively against intramolecular hydrogen bonds for entropoic reasons. However, the analine-like N*H* is not as acidic as 2-propanol, so the competition may be serious enough to interfere with chiral recognition.

The magnitudes of the separability factors for enantiomer separation on CSP 3 have always been noted to increase as the temperature is lowered. This is true in the present case ass well (Table III). Resolution of the two bands is enhanced as the temperature is lowered although there is accompanying band broadening. Consequently, the ability to resolve the two bands increases more slowly than does the separability factor.

ACKNOWLEDGEMENTS

We acknowledge the support of NSF Grant CHE 81-05030. The authors would like to thank Mr. John Kern, partially supported by the Syntex Corporation, for synthesizing compounds 1t–1z. We also would like to thank Dr. C. W. Rees of the Imperial College of Science and Technology, London, U.K., for supplying the indole from which indoline 2 g was derived.

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