Journal of Chromatography, 639 (1993) 329–332
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 075

Short Communication

Comments on a report of the separation of the enantiomers of π-donor analytes on π-donor chiral stationary phases

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(Received February 5th, 1993)

ABSTRACT

A report by Oliveros *et al.* [*J. Chromatogr.*, 589 (1992) 53] concerning the separation of the enantiomers of several π-basic analytes on π-basic chiral stationary phases was investigated. Claims for the separation of the enantiomers of two of these analytes are believed to be erroneous, the confusion in one instance presumably arising from the presence of an impurity in the commercially available material. Several methods for verifying the assignment of two chromatographic peaks as arising from enantiomers are also presented.

INTRODUCTION

A recent report by Oliveros *et al.* [1] concerns the separation of the enantiomers of compounds **1–8** (Fig. 1) using a series of π-donor chiral stationary phases (CSPs). Most of these CSPs are similar to analytes whose enantiomers are separable on N-(3,5-dinitrobenzoyl)amino acid-derived CSPs [2]. The reported separations of the enantiomers of the N-(3,5-dinitrobenzoyl)amino acid derivatives **1–3** (Fig. 1) on these CSPs comes as no great surprise given the reciprocal nature of chiral recognition. However, in our experience, π-donor CSPs typically show little or no ability to separate the enantiomers of π-donor analytes (unless the latter contain functionality capable of serving as hydrogen bond donors). It was thus with considerable interest that we read of the rather large separation factors ($\alpha > 20$ in one case) which were reported for the enantiomers of nitrile **7** and epoxide **8**, analytes which possess neither π-acidic functionality nor hydrogen bond donor groups. Such enantioselectivities suggested that some as yet undiscovered chiral recognition principle might be operative, and prompted us to further investigate some of these separations.

RESULTS AND DISCUSSION

From previous studies, we have available a column containing the naproxen-derived CSP first reported by Doyle *et al.* [3,4] and designated

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0021-9673/93/$06.00

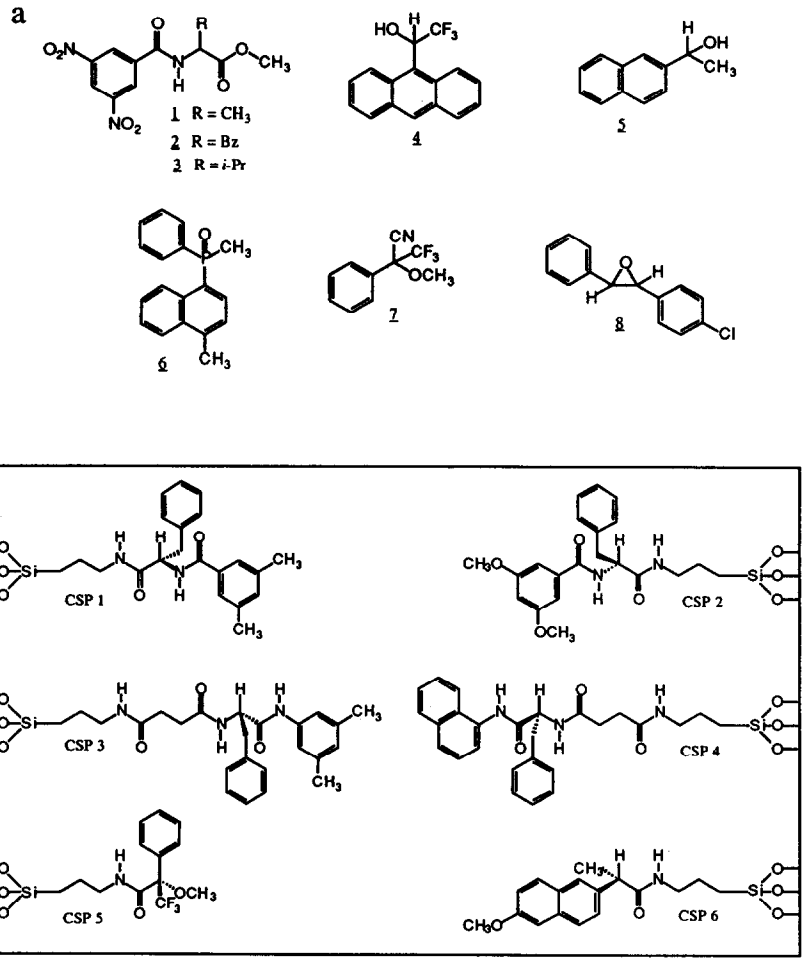
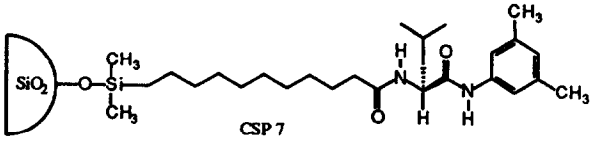


Fig. 1. (a) Analytes and (b) CSPs utilized in the study by Oliveros *et al.* [1].

as CSP 6 by Oliveros *et al.* We also have a column containing CSP 7, which is similar to CSP 3, the major differences being that CSP 7 is derived from leucine, whereas CSP 3 is derived from phenylalanine, and that CSP 7 is bonded to silica using a rather less complicated tether.



In our hands, the enantiomers of the ethyl ester of N-(3,5-dinitrobenzoyl)phenylalanine are separated on our CSP 6 ($\alpha = 1.94$, $k_1' = 0.85$) and CSP 7 ($\alpha = 2.46$, $k_1' = 0.79$) using the mobile phase described. These values compare favorably with the values reported for CSP 6 ($\alpha = 1.51$, $k_1' = 1.37$) and for CSP 3 ($\alpha = 2.10$, $k_1' = 0.65$) for the enantiomers of the methyl ester of N-(3,5-dinitrobenzoyl)phenylalanine, suggesting that our CSP 6 and CSP 7 are reasonable approximations of CSP 6 and CSP 3 used by Oliveros *et al.*

When samples of racemic nitrile **7** and racemic epoxide **8** were obtained from the vendor cited in the work by Oliveros *et al.* [1] and chromatographed on columns containing our CSP 6 and CSP 7, the results were inconsistent with the findings reported in the original study. Using the mobile phase reported by Oliveros *et al.* [1] (and several other mobile phases as well) nitrile **7**

affords but a single, scarcely retained peak. Epoxide **8** affords two peaks of comparable area on both CSP 6 and CSP 7. However, one of the peaks stems from an impurity since the same two peaks are noted on an achiral nitrile column. A trace of the more strongly UV absorbing 4-chlorostilbene, a logical precursor for **8**, was suspected to be present. However, gas chromatography–mass spectrometry shows the chlorine-containing impurity to have a molecular mass of 216/218 rather than the expected 214/216. The expected value of 230/232 was obtained for the epoxide. When epoxide **8** is chromatographed on a π-acidic CSP which *is* capable of separating its enantiomers [5], three peaks are observed: one giving a strong positive response from a polarimetric detector, one giving no response, and the last giving a strong negative response. No polarimetric response is noted when **8** is similarly chromatographed on CSP 6 or CSP 7.

Although we realize that preparation of the same CSP in two different laboratories will result in non-identical products, it seems likely that the separation factors of 3.55 and 4.20 reported for the enantiomers of nitrile **7** and the values 1.70 and 1.50 reported for epoxide **8** on CSPs 3 and 6 are erroneous. It seems likely that Oliveros *et al.* misinterpreted peaks arising from impurities as an indication of enantiomer separation when, in fact, no such separations were occurring.

One additional set of observations supports the view that the reported separations of the enantiomers of **7** are erroneous. On the strength of the large separation factors (*e.g.* 14.86, 23.00) they believed they were encountering on CSP 1 and CSP 2, Oliveros *et al.* prepared CSP 5 with the expectation that this reciprocal CSP would afford significant levels of enantioselectivity. In fact, in the series of analytes **1–8**, CSP 5 is reported to separate only the enantiomers of epoxide **8**. Since our sample of **8** (obtained from the same vendor as that of Oliveros *et al.*) is contaminated with an impurity, we suspect the claimed resolution of **8** by CSP 5 is also incorrect. The poor performance of CSP 5 was noted but no inference was drawn by Oliveros *et al.* from this observation.

Apparent separations of enantiomers are frequently encountered in chromatographic enantioseparation, particularly when multicomponent samples are investigated. Determining whether two chromatographic peaks arise from enantiomers can be facilitated by a variety of methods including:

(i) Use of a chiroptic detector. Many enantiomers can be detected using a polarimetric or circular dichroism detector, neither of which afford a response for a nonresolved racemate or an achiral sample.

(ii) Use of a racemic version of the CSP: the two peaks corresponding to the separated enantiomers typically show up as a single peak on a racemic version of the CSP [6]. In several instances [7,8] enantiofractionation has been observed during chromatography of enantioenriched analytes on an achiral CSP. However, this phenomenon is rarely encountered in practice. Chromatography of the sample on an achiral stationary phase (*e.g.* silica, amino, nitrile) affords a minimum estimate of the number of components in the sample, information relevant to the number of peaks to be expected with the CSP is used.

(iii) Use of a CSP of the opposite absolute configuration: this will invert the order of elution of the enantiomers, an event easily detected if the analyte is enantioenriched. This method is of no value for a racemic analyte unless used in conjuction with some other technique which is sensitive to stereochemistry (*e.g.* a chiroptic detector).

(iv) Use of a variable-wavelength detector: enantiomers have identical absorbtion spectra and must give identical detector responses. Relative peak heights of two supposed enantiomer peaks are therefore unaffected by changes in detector wavelength provided that an achiral mobile phase is used. The availability of modern full-spectrum diode array detectors makes this method especially useful.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Science Foundation, by a grant from Eli Lilly and Company, by a Department of Education Advanced Opportunities in Chemistry

Graduate Fellowhip to C.J.W. and by a generous donation of HPLC solvents from EM Scientific.

EDITORIAL NOTE

Dr. Laureano Oliveros, who is a co-author of ref. 1, has read the present paper and agrees that an experimental error was made in the study that is the subject of ref. 1. He has since been in contact with Dr. Pirkle in an effort to further clarify this situation.

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