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Journal of Chromatography A, 670 (1994) 239–242

JOURNAL OF CHROMATOGRAPHY A

Short Communication

Determination of enantiomeric purity of (*S*)-carboranylalanine using capillary column supercritical fluid chromatography

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(First received December 28th, 1993; revised manuscript received March 4th, 1994)

Abstract

Carboranylalanine, the *o*-carborane analogue of phenylalanine, is a potential candidate for boron neutron capture therapy of cancer. In this paper a method is described for the determination of enantiomeric purity of (*S*)-carboranylalanine as the (*N*-trifluoroacetyl)propylester using open tubular column supercritical fluid chromatography with a chiral stationary phase consisting of permethyl-β-cyclodextrin methyloctylsiloxane.

1. Introduction

Currently, much interest is being paid to the synthesis of compounds which could be used for boron neutron capture therapy (BNCT) of cancer [1–3]. α-Amino acids containing the boron-rich *closo*-1,2-$C_2B_{10}H_{11}$-carborane cage are potential candidates for this purpose [4,5]. (*S*)-Carboranylalanine (Fig. 1), the *o*-carborane analogue of phenylalanine, first reported in 1976 [6], was early shown to be able to replace phenylalanine in biological systems [7–9] and has recently attracted renewed interest for BNCT [5,10–12]. As the (*R*)- and (*S*)-form have different biological activity it is essential to find an accurate method for the determination of enantiomeric purity when working with biological applications [13].

In 1988 Harada and Takahashi [14] showed that the *closo*-1,2-$C_2B_{10}H_{12}$-carborane cage form strong inclusion complexes with cyclodextrins, a result indicating that cyclodextrins could be useful as chiral stationary phases (CSPs) for this type of compounds. Later Plešek *et al.* [15] managed to separate several enantiomers of zwitterionic deltahedral *nido*-carboranes and metallaboranes using liquid chromatography (LC) and CSPs based on cyclodextrins. These compounds owe their chirality to the dissymmetry of the cage. It was therefore tempting

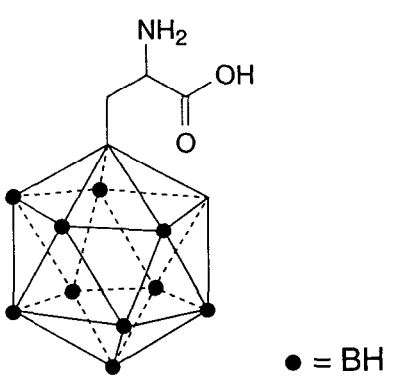

Fig. 1. (±)-Carboranylalanine.

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0021-9673/94/$07.00
SSDI 0021-9673(94)00221-T

to examine if a CSP based on permethylated β-cyclodextrin, which we have developed for open tubular column chromatography [16], is applicable to the separation of the enantiomers of carboranylalanine with a stereogenic centre in a side-arm of the carborane cage.

Unfortunately carboranylalanine is too polar to be eluted in gas chromatography (GC) or supercritical fluid chromatography (SFC) using carbon dioxide as mobile phase. It is also not readily analysed by LC or capillary electrophoresis since it only absorbs light at short wavelengths. An achiral derivatization is therefore motivated. As GC would give the highest efficiency it was decided to prepare the (N-trifluoroacetyl)propyl ester, however, no chiral selectivity was obtained by GC and a relatively high temperature was required to elute the enantiomers. The efforts were therefore focused on open tubular column SFC, a technique in which mainly the density of the mobile phase is used to control the retention of solutes and the temperature can therefore be kept at a favourably low level in order to improve the chiral selectivity as well as decrease the risk of thermal decomposition and racemization.

In the present work open tubular column SFC is used to investigate the enantiomeric purity of (*S*)-carboranylalanine obtained by asymmetric synthesis.

2. Experimental

The (N-trifluoroacetyl)propyl esters[a] of racemic [5] and (*S*)-carboranylalanine (obtained via alkylation of the Oppolzer's sultame-derived glycine equivalent [17] with propargylbromide, followed by reaction with decaborane and subsequent hydrolysis [18]) were prepared from the corresponding hydrochlorides by adapting the general procedure by Abe *et al.* [19]. A stock solution of hydrogen chloride in propanol was prepared by dropwise addition of 6 ml of acetyl chloride in propanol at 0°C and brought to room temperature after 10 min. The hydrogen chloride of the amino acid was dissolved in the acidic propanol solution (9 mg ml^{-1}) and heated at 100°C for 1 h. The solution was then evaporated to dryness and the residue pumped (*ca.* 0.1 mmHg, 13.3 Pa) for 4 h. Trifluoroacetic anhydride and dichloromethane (0.2 ml and 0.9 ml respectively, per 9 mg of amino acid hydrogen chloride) was added to the residue at 0°C. The mixture was heated to reflux for 15 min and then cooled to room temperature. The solution was evaporated to dryness, pumped at room temperature over night and the crystalline residue was homogenised.

In the preparation of sample and standard solutions dichloromethane was used as solvent. In order to minimize the evaporation of the solvent crimp-sealed vials were employed.

Chromatography was performed with a series 600-D supercritical fluid chromatograph (Dionex, Salt Lake City, UT, USA) equipped with a flame ionisation detector (350°C). The injector consisted of a CI4W.5 high-pressure four-port valve injector with a 0.5-μl sample loop (Valco Instruments, Houston, TX, USA) in combination with a splitter (split ratio *ca.* 1:10, 300 μm I.D.) (SGE, Austin, TX, USA). SFC grade CO_2 (Scott Speciality Gases, Plumsteadville, PA, USA) was used as mobile phase. The density of the mobile phase was kept constant along the column using a deactivated integral restrictor [20]. A 5 m × 50 μm I.D. deactivated open tubular column coated with a 53% (w/w) permethyl-β-cyclodextrin methyloctylsiloxane (film thickness *ca.* 0.25 μm) [16] was used for this study. Chromatograms were recorded with a SP4290 integrator (Spectra Physics, San Jose, CA, USA). The chromatograms were transferred from the integrator to a Macintosh IIfx computer (Apple Computer, Cupertino, CA, USA) and subsequently decoded with an in-house written routine in Microsoft QuickBasic (Microsoft, Redmond, WA, USA). A program for general graphing and data analysis, Igor

[a] ^{1}H-NMR data for the (*N*-tri-fluoroacetyl)propyl ester of carboranylalanine ($CDCl_3$): δ 7.06 (d, 1H, N*H*), 4.56 (dd, 1H, α-*H*), 4.16 (m, 2H, CH_2-O), 3.79 (bs, 1H, cage-H), 3.04 (dd, 1H, CH_2-CH), 2.77 (dd, 1H, CH_2-CH), 1.70 (m, 2H, CH_2-CH_3), 0.96 (t, 3H, CH_2-CH_3).

(Wave Metrics, Lake Oswego, OR, USA), was used for determination of peak areas.

3. Results and discussion

The separation was optimized to obtain baseline resolution, *i.e.* $R_s = 1.5$, within the shortest possible time (Fig. 2) [21]. As both the racemate and the sample containing the (*S*)-form and an unknown impurity of the (*R*)-form contained a small amount of impurities from the derivatization it was, unfortunately, not possible to utilize spiking to evaluate quantitatively the determination of enantiomeric purity without recrystallization.

In order to distinguish the peak corresponding to the (*R*)-form from the noise the column had to be overloaded and thus complete baseline resolution was no longer obtainable (the peak corresponding to the (*S*)-form becomes somewhat fronting) (Fig. 3). To decrease the influence of this partial overlapping and noise it

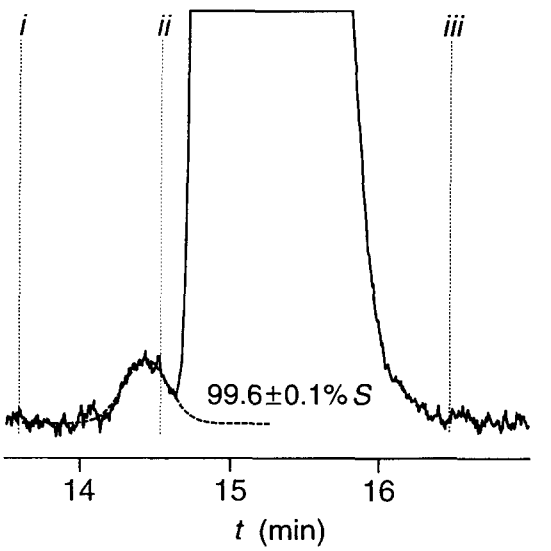

Fig. 3. SFC–FID chromatogram of the (*S*)-carboranylalanine sample (19.3 mg ml^{-1}). The dashed curve represents the fitted peak used for the area estimation of the minor peak. Enantiomeric purity of the (*S*)-form is given with a 95% confidence interval based on five measurements. Conditions: see Fig. 2.

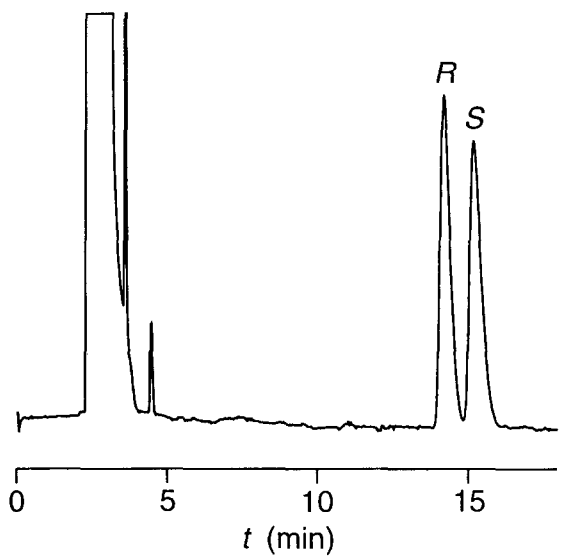

Fig. 2. SFC–FID chromatogram, the optimized separation of the (*N*-trifluoroacetyl)propyl ester of (±)-carboranylalanine (10.0 mg ml^{-1}). Conditions: 60°C, density programmed from 0.20 to 0.485 g ml^{-1} at 0.20 g ml^{-1} min^{-1} after a 2-min isopycnic period.

was decided to determine the area of the minor peak by fitting a Gaussian function [22,23] to the data points between *i* and *ii* in Fig. 3 after baseline subtraction. This should ensure a better area estimation than the more commonly used tangent skim or perpendicular drop. The total area of both peaks was subsequently estimated by trapezoidal integration between *i* and *iii* in Fig. 3.

Having repeated measurements of these areas it is then possible to estimate the enantiomeric purity of the sample. However, these calculations are only valid if the linearity of the detection system is ensured and the detector response to the different enantiomers is identical or known. Even though the flame ionization detector is known to be one of the most linear detectors available and it is not likely that the flame ionization detector should have different responses to the enantiomers it was decided to verify this. Five racemic standard solutions with concentrations covering the smallest and largest concentrations of the analytes in the sample were

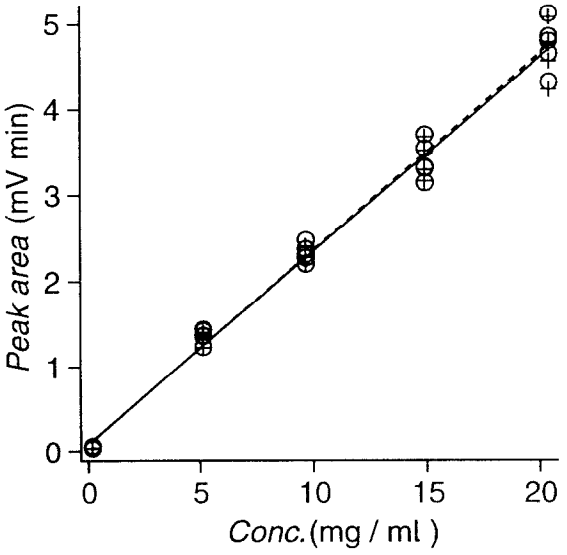

Fig. 4. Detector linearity and response, peak area *vs.* concentration for each enantiomer. The dashed line represents the (*R*)-form (○) and the solid line the (*S*)-form (+). The regression coefficient was 0.996 for both forms.

each injected five times. As shown in Fig. 4 the linearity is good and there is no significant difference in detector response, in other words, the method should be valid. The increasing standard deviation of peak area with increasing concentration is related to the injection technique which is expected to give a relative standard deviation in the order of 5%.

The enantiomeric purity of the (*S*)-form, defined as the percentage of the ratio between the area of the peak corresponding to the (*S*)-form and the sum of peak areas of both forms, was found to be 99.6 ± 0.1% (95% confidence interval based on five measurements). This result supports the suggested method for quantification as well as the method for asymmetric synthesis of (*S*)-carboranylalanine [18].

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