Journal of Chromatography, 112 (1975) 197–202

CHROM. 8520

METHIONINE DIPEPTIDE STATIONARY PHASES FOR THE RESOLUTION OF ENANTIOMERS

F. ANDRAWES, R. BRAZELL, W. PARR and A. ZLATKIS
Chemistry Department, University of Houston, Houston, Texas 77004 (U.S.A.)

SUMMARY

N-Trifluoroacetyl-L-methionyl-L-methionine cyclohexyl ester as well as the disulfide and disulfone derivatives of this phase have been synthesized. The methionine phase exhibits improved properties as a stationary phase for the separation of amino acid enantiomers as compared to other dipeptide stationary phases and can be used at temperatures ranging from 70 to 150°. The sulfoxide phase, however, was incapable of resolving the enantiomeric derivatives. Physical properties of the sulfone compound limit its use as an effective resolving agent.

INTRODUCTION

Of the two methods[1,2] that are available at the present time for the separation of amino acid enantiomers by gas–liquid chromatography, the method involving the direct separation on optically active phases[2–9], has proven to be the most reliable. Further, it has been found that N-trifluoroacetyl (TFA)-L,L-dipeptide cyclohexyl esters are the most suitable stationary phases[3–9]. These phases, however, have limited thermal stability and retention times are relatively long.

Since the advent of the first optically active dipeptide stationary phase[2], a number of different dipeptide phases were prepared and examined[3–9]. The mechanism of the separation has been studied[4,6] and the effect of the length and the branching on the side chain of solute and solvent on the separation was also investigated[7]. To date, however, none of the dipeptides examined contained sulfur on the side chain. In the course of this study, three new phases containing sulfur were prepared: methionine dipeptide phase, as well as the disulfoxide and the disulfone phases. These latter compounds were prepared to examine the effect of increasing bulkiness and polarity on solvent–solute interaction, as well as the effect on thermal stability.

EXPERIMENTAL

Synthesis of N-TFA-L-methionyl-L-methionine cyclohexyl ester

L-Methionine cyclohexyl ester was prepared as previously described[7]. Yield, 95%. M.p., 93–94°.

N-TFA-L-methionine was synthesized according to the procedure of Weygand and Geiger[12]. Yield, 94%. M.p., 80–82°.

N-TFA-L-methionyl-L-methionine cyclohexyl ester was synthesized as follows. 10 mmoles of L-methionine cyclohexyl ester hydrogen chloride were dissolved in 10 ml of tetrahydrofuran (THF). The solution was neutralized with 10 mmoles of triethylamine. 10 mmoles of N-TFA-L-methionine were dissolved in 15 ml THF, and 11 mmoles of N,N'-carbonyldiimidazole were dissolved in 10 ml of THF. The three solutions were combined and stirred at room temperature for 12 h, after which the mixture was filtered and the solvent evaporated. The remaining clear oil was dissolved in ethyl acetate and then extracted once with a 10% solution of sodium bicarbonate and once with a 2 *N* solution of citric acid. It was then extracted with distilled water until neutral. The organic layer was dried over anhydrous sodium sulfate and excess solvent was removed *in vacuo*. The remaining oil was further purified by column chromatography over neutral alumina using an LKB ultrorac (R) Model 7000 fraction collector and collecting fractions absorbing at 254 nm. The final product was a clear oil. Yield, 86%. The structure was confirmed by infrared (IR) spectroscopy, mass spectroscopy and elemental analysis.

Synthesis of N-TFA-L-methionyl sulfoxide-L-methionine sulfoxide cyclohexyl ester

L-Methionine sulfoxide was prepared from L-methionine according to Toennies and Kolb[10]. N-TFA-L-methionine sulfoxide and L-methionine sulfoxide cyclohexyl ester were prepared and coupled as mentioned for the methionine phase. The yield utilizing this method was low, however, and a better way to synthesize this compound was found to be the oxidation of the methionine dipeptide phase: 10 mmoles of N-TFA-L-methionyl-L-methionine cyclohexyl ester were dissolved in 60 ml of methanol and then cooled in an ice-bath. 18 mmoles of concentrated hydrochloric acid at 0° were added and 24 mmoles of hydrogen peroxide (30% solution) were added. The mixture was then stirred in an ice-bath for 10 min and then for 30 min at room temperature. It was neutralized with a 10% solution of sodium bicarbonate. Filtration followed, and the solvent was removed *in vacuo*. The remaining oil was dissolved in ethyl acetate and then extracted three times with water. The ethyl acetate was evaporated and the remaining oil was further purified by column chromatography as described above. The product was a clear oil. Yield, 78%. Attempts to crystallize the compound using different solvents were unsuccessful. The structure was verified by IR and elemental analysis.

Synthesis of N-TFA-L-methionyl sulfone-L-methionine sulfone cyclohexyl ester

The oxidation of the methionine dipeptide phase was found to be a better method of preparation than the coupling of N-TFA-L-methionine sulfone and methionine sulfone cyclohexyl ester. The oxidation was carried out as follows: 10 mmoles of N-TFA-L-methionyl-L-methionine cyclohexyl ester were dissolved in 50 ml of methanol and 5 ml of 5 *M* perchloric acid. 2 ml of 0.5 *M* ammonium molybdate were added, followed by 60 mmoles of hydrogen peroxide (5 ml of 30% hydrogen peroxide). The solution was cooled in an ice-bath and the white precipitate which formed on the addition of the molybdate dissolved when the hydrogen peroxide was added, resulting in a yellow solution. The mixture was stirred at 0° for 20 min, and then for 1.5 h at room temperature. The mixture was diluted with methanol periodically because

precipitating sulfone made stirring difficult. After the reaction was completed the sulfone phase was filtered and washed with a 10% solution of sodium bicarbonate, and then with water until neutral. The compound was dried under vacuum at 60° for 3 h. Yield, 90%. M.p., 181–184°. The sulfone phase was not soluble in common solvents. The structure was verified by IR and elemental analysis.

Preparation of N-TFA-D,L-amino acid isopropyl esters

The derivatives were prepared according to a method reported previously[7].

Gas chromatography

Analyses were carried out on a Varian 1200 gas chromatograph equipped with a flame ionization detector. Stainless-steel capillary columns (100 and 135 m × 0.5 mm I.D.) were used for the analysis. The columns were coated with 10% solutions of the phases in methylene chloride and conditioned for 24 h at 160° while helium carrier gas was passed through at 6 p.s.i.

RESULTS AND DISCUSSION

N-TFA-L-methionyl-L-methionine cyclohexyl ester

This dipeptide phase was prepared in high yield (86%) in only three steps. When the compound was examined as a stationary phase, it was found that it could be used adequately at temperatures ranging from 70 to 150°. This wide range makes it possible to utilize temperature programming. Fig. 1 shows a temperature-programmed chromatogram for 10 pairs of amino acid enantiomeric derivatives. Separation factors ($\alpha_{L/D}$) and relative retention times have been calculated from the chromatogram shown in Fig. 1 and are given in Table I. These separation factors, however, vary with changing conditions. Fig. 2 shows a baseline separation for three pairs of enantiomers in 35 min indicating high resolving ability.

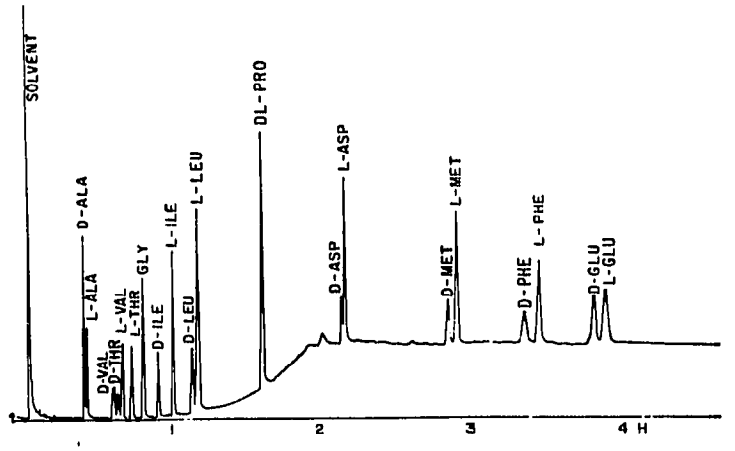

Fig. 1. Chromatogram of N-TFA-D,L-amino acid isopropyl esters with N-TFA-L-methionyl-L-methionine cyclohexyl ester as stationary phase. Conditions: 100 m × 0.5 mm I.D. stainless-steel capillary column, operation at 70° isothermal for 30 min, then temperature-programmed 0.5°/min to 110°; injector temperature, 180°; detector temperature, 275°; carrier gas, helium at 20 p.s.i.

TABLE I

RELATIVE RETENTION TIMES AND SEPARATION FACTORS FOR N-TFA-D,L-AMINO ACID ISOPROPYL ESTERS ON N-TFA-L-METHIONYL-L-METHIONINE CYCLOHEXYL ESTER AS STATIONARY PHASE

Amino acid	*Relative retention time**	*Separation factor*
D-Alanine	0.320	1.037
L-Alanine	0.334	
D-Valine	0.495	1.078
L-Valine	0.544	
D-Threonine	0.518	1.131
L-Threonine	0.602	
Glycine	0.667	
D-Isoleucine	0.761	1.103
L-Isoleucine	0.845	
D-Leucine	0.962	1.037
L-Leucine	1.000	
D-Proline	1.374	1.00
L-Proline	1.374	
D-Aspartic acid	1.840	1.010
L-Aspartic acid	1.868	
D-Methionine	2.476	1.024
L-Methionine	2.528	
D-Phenylalanine	2.919	1.027
L-Phenylalanine	2.997	
D-Glutamic acid	3.317	1.022
L-Glutamic acid	3.389	

* Relative retention time with respect to L-leucine.

As can be seen from the temperature-programmed run (Fig. 1) proline was not resolved. This amino acid, however, has proven to be difficult to resolve on other dipeptide stationary phases as well[6,9]. Leucine and serine overlap although the D- and L-forms of each are well resolved when the amino acids are injected singly. The overlapping problem, however, can be solved by coupling the dipeptide stationary phase column to a 30 m × 0.5 mm I.D. column coated with OV-17 (ref. 9).

The phase was thermally stable at temperatures as high as 150°. Increasing temperatures, however, reduce the separation factors and at 140° there was no resolution for D,L-alanine of D,L-valine. Above 150° the column began to bleed. Among all N-TFA dipeptide cyclohexyl ester stationary phases only the methionine and the phenylalanyl-leucine phase exhibit thermal stability at 150°. Most other such phases bleed above 110° (refs. 3 and 8). The thermal stability of the dipeptide stationary phases is of interest since one may prepare a dipeptide stationary phase which can be successfully operated at elevated temperatures but is unable to resolve enantiomers. This may be attributed to the disruption of the diastereoisomeric association complex (Fig. 3) as the temperature is increased.

The fact that the diastereoisomeric association complex is disturbed at 140° for alanine and valine in the methionine phase and not disturbed in the phenylalanyl-leucine phase is of interest. It may be that a specific conformation is required in order for the diastereoisomeric association complex to take place. In the methionine phase

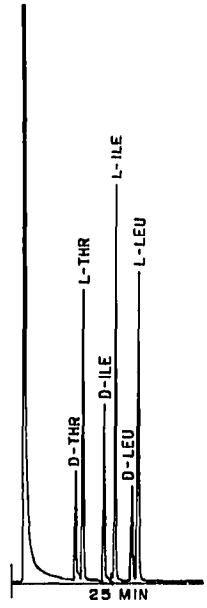

Fig. 2. Chromatogram of N-TFA-D,L-threonine, isoleucine, and leucine isopropyl esters on N-TFA-L-methionyl-L-methionine cyclohexyl ester as stationary phase. Conditions: 100 m × 0.5 mm I.D. stainless-steel capillary column, 90° isothermal operation; injection temperature, 185°; detector temperature, 275°; carrier gas, helium at 20 p.s.i.

this conformation can be changed at relatively low temperatures since the side chain on the asymmetric carbon is straight, while the side chain of the phenylalanyl-leucine phase has a phenyl group which hinders the motion of the molecule. Higher temperatures are therefore required to change the proper conformation in this latter phase.

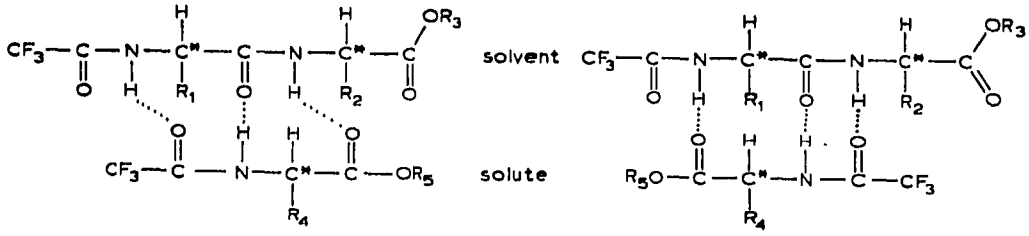

Fig. 3. Diastereoisomeric association complex.

N-TFA-L-methionyl sulfoxide-L-methionine sulfoxide cyclohexyl esters

The phase was prepared in high yield (78 %) via the oxidation of the methionine phase. However, when the compound was examined as a gas chromatographic stationary phase on a capillary column (135 m × 0.5 mm I.D.), no resolution for enantiomers was obtained. It is possible that the sulfoxide is intramolecularly hydrogen-bonded to the amide group as shown in Fig. 4. Evidence for this is given as follows:

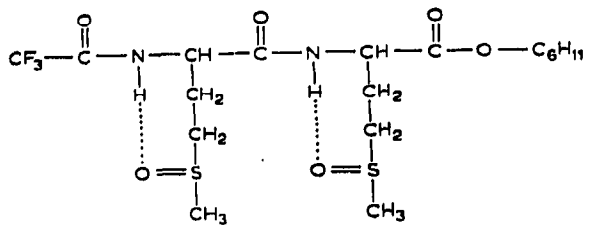

Fig. 4. Possible structure showing hydrogen bonding between the sulfoxide and the hydrogen of the amide.

(1) Stretching of the sulfoxide bond in the IR spectrum shows a band in the range of 1040–1060 cm^{-1}. This shifts to lower values when hydrogen bonded[11]. The sulfoxide bond (in 10% solution) of the sulfoxide phase is shifted 25 cm^{-1} (1015 cm^{-1}) (ref. 11), indicating hydrogen bonding.

(2) IR spectroscopy for very dilute solutions (1%) of the sulfoxide phase showed that the sulfoxide bond did not change positions, indicating that the hydrogen bonding is intrahydrogen bonding.

(3) The polarity of the phase is reduced as compared to that of the methionine phase. The retention time is approximately 1/2 of that of the methionine phases at the same conditions.

*N-TFA-*L*-methionyl sulfone-*L*-methionine sulfone cyclohexyl ester*

The phase was synthesized in high yield (90%). The high melting point of the compound (181–184°) and its insolubility in any of the proper solvents rendered it unsuitable for use as a stationary phase.

ACKNOWLEDGEMENT

We gratefully acknowledge the support of this work by the National Aeronautics and Space Administration, Life Sciences Directorate, Johnson Space Center, Houston, Texas (Contract NAS 9-13457).

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