Journal of Chromatography, 150 (1978) 207–211

CHROM. 10,536

Note

Selection of a stable liquid stationary phase for the gas–liquid chromatographic separation of small peptide derivatives

FARZANEH MOHAMMADI-TABRIZI*

Robert Robinson Laboratories, Liverpool University, Liverpool L69 3BX (Great Britain)

(First received January 20th, 1977; revised manuscript received July 21st, 1977)

Peptide mixtures are difficult to separate, although various methods have been used to separate simple amino acids and small peptides from large peptides[1–10]. In gas–liquid chromatography (GLC), the sample compounds must be volatile at the operating temperature; peptides, because of their zwitterionic nature and the strong intermolecular hydrogen bonding between the amide bonds, are not sufficiently volatile and hence present problems on passing through a GLC column. Several derivatives have been used, but difficulties arise in separating some of them because of the increased possibility of isomerism. Therefore, it is not satisfactory simply to measure retention times in order to identify peptides; each peak from a GLC column must be investigated by some other technique, and mass spectrometry is very useful for this purpose.

In this work, we investigated the GLC of peptide derivatives, using as the liquid stationary phase OV-1, OV-17, PolyMPE and Dexsil 300 GC, followed by mass spectrometry.

EXPERIMENTAL

Numerous samples of peptides and peptide methyl esters were received locally from the peptide synthesis laboratories.

Derivatization

Peptide derivatives other than the above methyl esters were prepared as follows. The N-terminal acetyl and trifluoroacetyl derivatives were prepared with acetic acid–acetic anhydride (1:1) and trifluoroacetic acid–trifluoroacetic anhydride (1:2), respectively, as described by Senn *et al.*[11]. The N-permethylation of peptide derivatives was carried out with sodium hydride–dimethylformamide–methyl iodide (5:2:5 mmole for every NH group in the peptide)[12, 13].

* Present address: Institute of Nuclear Science and Technology, Tehran University, P.O. Box 2989, Tehran, Iran.

Gas–liquid chromatography

Individual peptide derivatives and their mixtures were chromatographed on a Pye Model 104 provided with a flame-ionization detector using nitrogen as the carrier gas. The temperature of the injection block was always about 50° higher than that of the column. To collect samples for mass spectrometry, a simple splitter was used at the column exit, and the emerging peptide derivative was condensed in a capillary tube that could be transferred to the mass spectrometer without further handling. Other experimental conditions are given in the tables and figure captions.

The mass spectra of the peptide derivatives were determined on an A.E.I. MS902 or MS12 mass spectrometer using an accelerating voltage of 8 kV, a beam energy of 70 eV and a trap current of 500 μA. The source temperature was usually about 220°.

RESULTS AND DISCUSSION

In determining the best liquid stationary phase for these separations, stability at high temperatures and resolving efficiency were considered. Columns packed with OV-1 or Dexsil had a better efficiency and gave shorter retention times in separating non-polar peptide derivatives (Table I). Also, these phases had optimal separation properties at lower temperatures. The retention times should be treated with caution. For example, at 205° the retention times of the dipeptide CH_3CO.(Me)ala.(Me)phe.OMe on columns packed with OV-1, PolyMPE or Dexsil are 11, 53 and 23 min, respectively. The separation efficiency as measured by the height equivalent to a theoretical plate (HETP) is good for Dexsil, but PolyMPE, which gives longer retention times, has relatively poor HETP characteristics. The HETP of the column determines the length of time needed for a substance to be eluted from the column, and is important in connection with column bleeding as contamination of the sample with the liquid phase will complicate the identification of the peptides in the subsequent mass spectrum. The HETP values for the various liquid phases at different operating temperatures are shown in Table II.

TABLE I

RETENTION TIMES OF Ac.(Me)ala.(Me)phe.OMe AT VARIOUS TEMPERATURES ON DIFFERENT LIQUID STATIONARY PHASES

Carrier gas: nitrogen, flow-rate 45 ml·min^{-1}. Columns: 7 ft. packed with 5% OV-1, Dexsil 300 GC, OV-17 and PolyMPE.

Oven temperature (°C)	*Retention times (min)*			
	OV-1	*Dexsil*	*OV-17*	*PolyMPE*
300	1.5	2		2.5
285		3.33		3.71
280			6	
270	2.5		8.5	5.5
255	3	6	14	9
240			21.33	14
225	6	13	51.33	25.25
205	11	23		53
190	19	43		

TABLE II

HETP VALUES FOR DIFFERENT LIQUID PHASES FOR THE DIPEPTIDE CH_3CO.(Me)ala.-(Me)phe.OMe AT VARIOUS OPERATING TEMPERATURES

Liquid phase	*Operating temperature (°C)*	*HETP*	*Length of column (ft.)*	*Amount of liquid phase (%)*
OV-17	240	329	7	5
OV-17	255	366	7	5
OV-17	270	329	7	5
OV-17	285	269	7	5
OV-1	190	98	7	5
OV-1	205	77	7	5
OV-1	255	54	7	5
PolyMPE	205	68	5	4
PolyMPE	225	127	5	4
PolyMPE	240	282	5	4
PolyMPE	290	205	5	4
Dexsil 300 GC	205	291	7	15
Dexsil 300 GC	225	285	7	15
Dexsil 300 GC	250	177	7	15
Dexsil 300 GC	275	171	7	15

In these experiments, Dexsil had the best stability at high temperatures and the highest resolving efficiency, and gave the best results in separating peptides at as low a temperature as is practically feasible.

In selecting columns for GLC separations, the "polarity" of a column is dependent not only on the stationary phase but also on the substance being analysed[14]. Therefore, several derivatives of a peptide were chromatographed and their retention times were determined (Table III). Although the retention times and resolutions were good for polar peptide derivatives, they were found to condense very readily on leaving the GLC column. Further, the mass spectra of these derivatives were not very intense and well defined fragmentation patterns were not obtained for many types of peptides. The most suitable derivatives were the N-acetyl peptide methyl esters.

In order to cover a wide range of volatilities in a peptide mixture, temperature programming was used. In the gas chromatograms of derivatized partial acid hydrolysates of tyrocidine and a decapeptide (Figs. 1 and 2) some small peptides were

TABLE III

RETENTION TIMES OF PEPTIDE DERIVATIVES AT 250° ON 5% OV-1 AND DEXSIL COLUMNS

Carrier gas: nitrogen, flow-rate 45 ml·min^{-1}. Columns: 7 ft.

Peptide derivative	*Retention times (min)*	
	OV-1	*Dexsil*
H.ala.phe.leu.OMe	11.2	1.33
CF_3CO.ala.phe.leu.OMe	10.83	11
CH_3CO.ala.phe.leu.OMe	22.2	25.83
CH_3CO.(Me)ala.(Me)phe.(Me)leu.OMe	26.13	26.2

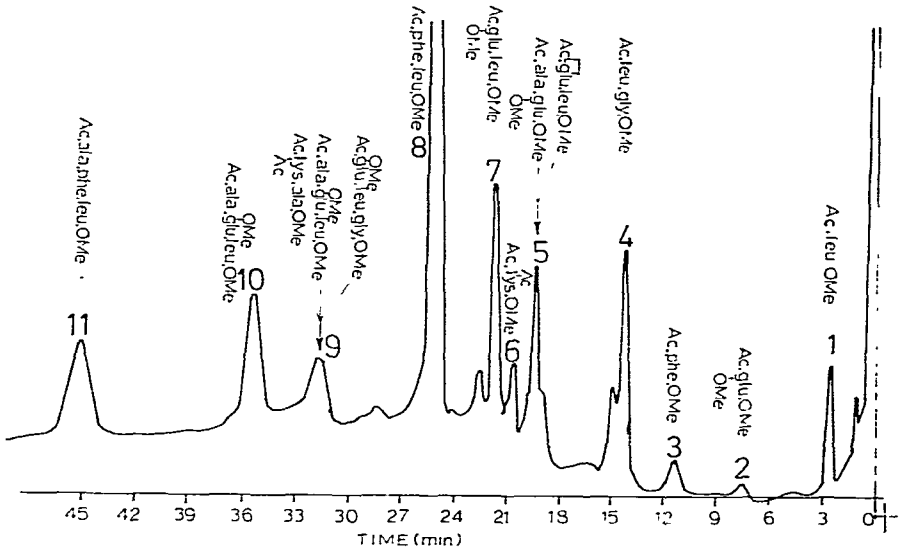

Fig. 1. Gas chromatogram of small peptides obtained by hydrolysis of the octapeptide Z.ala.glu.leu.-gly.lys.ala.phe.leu.OMe on 15% Dexsil (7 ft. column) at 195–295° (programmed at 6°·min^{-1} after an initial delay of 5 min). Nitrogen flow-rate, 45 ml·min^{-1}.

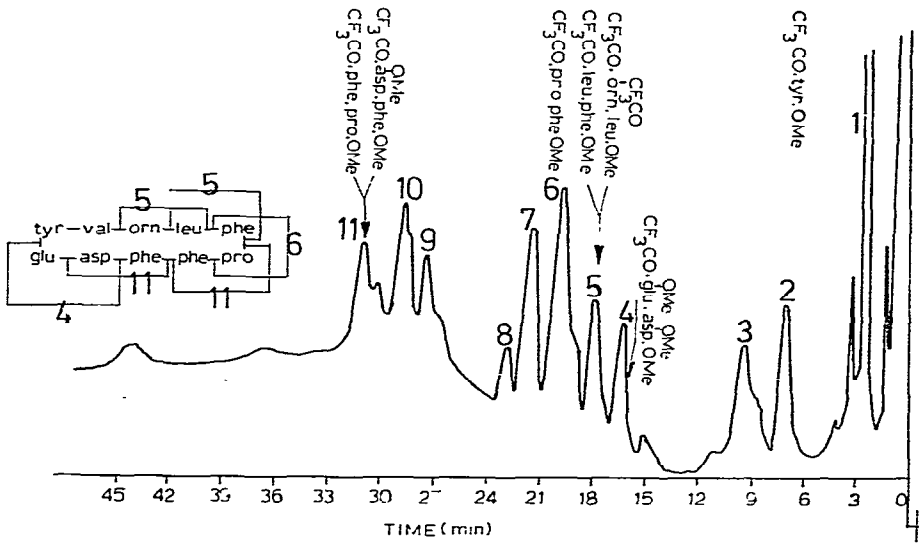

Fig. 2. Gas chromatogram of the small peptides obtained by hydrolysis of tyrocidine on 7% Dexsil (5 ft. column) at 195–270° (programmed at 6°·min^{-1} after an initial delay of 5 min). Nitrogen flow-rate, 45 ml·min^{-1}.

identified from the samples eluted from the Dexsil column by recording and interpreting the mass spectral data.

ACKNOWLEDGEMENTS

The author expresses her gratitude to Professor G. W. Kenner for the facilities made available during this research. Appreciation is also extended to Dr. R. A. W. Johnstone for his encouragement. This work was carried out with financial assistance from the Central Treaty Organization, who provided a Fellowship.

REFERENCES

1 E. Gelpi, W. A. Konig, J. Gilbert and J. Oro, *J. Chromatogr. Sci.*, 7 (1969) 604.
2 W. A. Konig, W. Parr, H. A. Lichtenstein, E. Bayer and J. Oro, *J. Chromatogr. Sci.*, 8 (1970) 183.
3 S. Nakaparksin, P. Birrell, E. Gil-Av and J. Oro, *J. Chromatogr. Sci.*, 8 (1970) 177.
4 Z. Deyl, *J. Chromatogr.*, 127 (1976) 91.
5 W. Parr and P. Y. Howard, *Anal. Chem.*, 45 (1973) 711.
6 R. Brazell, W. Parr, F. Andrawes and A. Zlatkis, *Chromatographia*, 9 (1976) 57.
7 R. A. Hites and K. Biemann, *Anal. Chem.*, 40 (1968) 1217.
8 G. Hudson and K. Biemann, *Biochem. Biophys. Res. Commun.*, 71 (1976) 212.
9 M. Makita, S. Yamamoto and M. Kōno, *J. Chromatogr.*, 120 (1976) 129.
10 W. A. König and W. Rahn, *J. Chromatogr.*, 133 (1977) 141.
11 M. Senn, R. Venkataraghavan and F. W. McLafferty, *J. Amer. Chem. Soc.*, 88 (1966) 5593.
12 D. W. Thomas, *FEBS Lett.*, 5 (1969) 53.
13 G. Marino, L. Valente, R. A. W. Johnstone, F. M. Tabrizi and G. C. Sodini, *Chem. Commun.*, (1972) 357.
14 G. Tarján, Á. Kiss, G. Kocsis, S. Mészáros and J. M. Takács, *J. Chromatogr.*, 119 (1976) 327.