CHROM. 3688

Gas–liquid chromatography of diketopiperazines

Dipeptides have been separated by a variety of chromatographic procedures, including GLC of their N-trifluoroacetyl methyl esters[1–4]. Separation of dipeptides into diastereoisomers has been observed during paper[5] and thin-layer[6] chromatography, and also by GLC[1,3,4]; the latter observation is the basis for a method[3] for optical resolution of amino acids by GLC via dipeptide formation. In contrast, the chromatography of *cyclic* dipeptides (diketopiperazines) has received little attention, although an excellent thin-layer chromatographic system has been described[7]. Only one limited report[2] on GLC has appeared, in which a silicone column gave strongly asymmetric peaks and no separation of diastereoisomers. In the work reported here, symmetrical peaks were obtained with good diastereoisomeric separation in most cases. Application of the method to an investigation of diketopiperazine formation during thermal degradation of peptides will be described elsewhere.

Apparatus

A Glowall model 310 gas chromatograph equipped with a flame ionization detector and a Honeywell recorder was used, with coiled glass columns as follows: column A, 6 ft. × 3.4 mm of 3 % EGSP-Z on Gas Chrom Q, 100–120 mesh; column B, 3 ft. × 3.4 mm of 3 % SE-30 on Gas Chrom Q, 100–120 mesh. Carrier gas: argon at 40 ml/min.

Materials

The diketopiperazines containing alanine and phenylalanine were kindly supplied by WESTLEY*. Others were synthesised by published methods[8]; details of previously unknown compounds will be reported elsewhere. In some cases, diastereoisomeric mixtures were prepared from the *cis* (L-L) isomer by equilibration for 30 min in boiling 0.3 *N* methanolic sodium methoxide, a procedure which afforded mixtures containing mainly the *trans* isomer. Most of the N-methyl compounds were prepared by methylation[9] of the requisite parent diketopiperazine. Methanol or dimethylformamide were used as solvents for injection.

N,N'-Bis(trimethylsilyl) (TMS) derivatives[10] were typically prepared as follows: the diketopiperazine (1–5 mg) in a 25 % solution (0.2–1.0 ml) of N,O-bis(trimethylsilyl) acetamide in dry dimethylformamide was heated for 10 min at 80° in a capped vial. An aliquot of the resulting solution, which contained excess reagent, was injected directly into the gas chromatograph.

Results

Retention times of various diketopiperazines on column A at two temperatures, and of the relatively volatile N-methyl compounds at the lower temperature, are given in Table I. Narrow, symmetrical peaks were obtained and essentially similar separations were observed with a column of 1 % EGSP-Z on Gas Chrom P, on which retention times were approximately halved. A 3 % NGS column was inferior for this purpose.

* Department of Genetics, Stanford University School of Medicine, Palo Alto, Calif., U.S.A.

TABLE I

RETENTION TIMES (min) OF DIKETOPIPERAZINES ON COLUMN A

Diketopiperazine	*206°*	*196°*	*N-Methyl-diketopiperazine*	*196°*
Gly-Gly	25.3	41.5	Sar-Sar	5.8
Gly-Ala	15.5	25.6	Sar-Val	9.5
Gly-Val	17.1	28.7	Sar-Meval	5.3
Gly-Pro	22.8	37.5	Sar-Pro	13.8
Ala-Val (*c*+*t*)	10.4	17.4	Sar-Cycloleu[a]	17.2
Ala-Leu (*c* + *t*)	13.4	22.1	Val-Meval (*c*)	10.5
Val-Val (*c*)	10.6	17.2	Val-Meval (*t*)	7.0
Val-Val (*t*)	9.6	15.4	Meval-Meval (*c*)	5.4
Val-Pro (*c*)	12.3	19.5	Meval-Meval (*t*)	3.5
Val-Pro (*t*)	16.7	26.8		
Ileu-Pro (*c*)	16.3	25.7		
Ileu-Pro (*t*)	21.8	35.2		

[a] "Cycloleu" refers to aminocyclopentane-1-carboxylic acid[11].

TABLE II

RETENTION TIMES OF TMS-DIKETOPIPERAZINES ON COLUMN B

Diketopiperazine	*Temperature*	*Retention time*
Gly-Gly	115°	4.1
Ala-Val (*c*)	115°	5.4
Ala-Val (*t*)	115°	4.9
Ala-Leu (*c*+*t*)	115°	10.6
Val-Val (*c*)	115°	11.3
Val-Val (*t*)	115°	9.7
Gly-Phe	165°	4.5
Ala-Phe (*c*)	165°	4.9
Ala-Phe (*t*)	165°	4.5
Leu-Phe (*c* + *t*)	165°	8.0

Column A gave a base-line separation of the *cis* (L-L or D-D) and *trans* (D-L) forms of valine diketopiperazine. An even higher degree of diastereoisomeric resolution was observed for valyl-prolyl and the structurally similar isoleucyl-prolyl diketopiperazines; retention time ratios (*trans/cis*) were 1.37 in each case. In contrast, this column failed to distinguish the diastereoisomers of alanyl-valyl and alanyl-leucyl diketopiperazines.

Retention times of some N-trimethylsilyl diketopiperazines on column B are given in Table II. The diketopiperazines containing phenylalanine required a higher temperature for GLC than the purely aliphatic ones.

Discussion

On the EGSP-Z column the diketopiperazines under study displayed a wide range of retention times, shortest for those containing alanine or valine and longest for those in which glycine or proline was present. N-Methylation drastically reduced retention times. The degree of diastereoisomeric separation varied greatly, being

highest for diketopiperazines containing proline or N-methylvaline and zero for those containing alanine.

N-Trimethylsilylation permitted the satisfactory use of a column of the silicone type, on which retention times were comparatively dependent upon molecular weight. Diastereoisomeric separation was again encountered, but for the diketopiperazines composed of simple aliphatic amino acids the use of column A without derivatization was generally superior. For less volatile diketopiperazines, however, the advantage of trimethylsilylation is obvious, since it permits GLC on silicone columns without "tailing". The diketopiperazines containing phenylalanine could not be chromatographed on column A, but on column B, after trimethylsilylation, convenient retention times were obtained at a temperature far below maximum. It follows that GLC may be applicable to even less volatile diketopiperazines by this means, since additional functional groups would also be trimethylsilylated. This possibility is under experimental study.

Applications of the techniques described here in the areas of peptide chemistry and natural products are under investigation.

Acknowledgements

The gift of several diketopiperazines by Dr. J. W. WESTLEY, the technical assistance of Mrs. I. RITTNER, and the support of grant No. AI 07662 from the National Institutes of Health are gratefully acknowledged.

Research Foundation of the Washington Hospital Center, 110 Irving Street, Northwest, Washington, D. C. 20010 (U.S.A.)

ANTHONY B. MAUGER

1 F. WEYGAND, B. KOLB, A. PROX, M. A. TILAK AND I. TOMIDA, *Z. Physiol. Chem*, 322 (1960) 38.
2 F. WEYGAND, A. PROX, E. C. JORGENSEN, R. AXEN AND P. KIRCHNER, *Z. Naturforsch.*, 18 (1963) 93.
3 B. HALPERN AND J. W. WESTLEY, *Biochem. Biophys. Res. Commun.*, 19 (1965) 361.
4 F. WEYGAND, A. PROX, L. SCHMIDHAMMER AND W. KONIG, *Angew. Chem. (Intern. Ed.)*, 2 (1965) 183.
5 B. RINIKER AND R. SCHWYZER, *Helv. Chim. Acta*, 44 (1961) 658;
J. W. HINMAN, E. L. CARRON AND H. N. CHRISTENSEN, *J. Am. Chem. Soc.*, 72 (1950) 1620.
6 T. WIELAND AND E. BENDE, *Chem. Ber.*, 98 (1965) 504.
7 D. E. NITECKI, B. HALPERN AND J. W. WESTLEY, *J. Org. Chem.*, 33 (1968) 864.
8 E. FISCHER, *Chem. Ber.*, 39 (1906) 2893;
K. D. KOPPLE AND H. G. GHAZARIAN, *J. Org. Chem.*, 33 (1968) 862.
9 J. F. KLEBE, H. FINKBEINER AND D. M. WHITE, *J. Am. Chem. Soc.*, 88 (1966) 3390;
K. RUHLMAN AND B. RUPPRICH, *Ann.*, 686 (1965) 226.
10 B. C. DAS, S. D. GERO AND E. LEDERER, *Biochem. Biophys. Res. Commun.*, 29 (1967) 211.
11 T. A. CONNORS, A. B. MAUGER, M. A. PEUTHERER AND W. C. J. ROSS, *J. Chem. Soc.*, (1962) 4601.

Received July 8th, 1968