Journal of Chromatography, 115 (1975) 633–634

CHROM. 8672

Note

Separation of dinitrophenyl derivatives of neutral dipeptides by thin-layer chromatography

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(Received July 16th, 1975)

In the course of a problem involving dipeptide synthesis, we were required to find a simple method for verifying amino acid analyzer peaks of oligopeptides. This is generally easily accomplished with standards. However, in this case of N-terminal valyl peptides, the response to the normal ninhydrin reagent is small and therefore peaks of low intensity may be missed. Heathcote *et al.*[1] recently solved this problem for a number of dipeptides using two-dimensional thin-layer chromatography and a ninhydrin–cadmium acetate reagent for visualizing peptides. We considered that dinitrophenyl (DNP) derivatives of the dipeptides might also be well separated and would give no problem due to ninhydrin response because of the intense yellow colour of the derivatives. As far as we are aware, the only study of DNP dipeptides does not include TLC data[2]. Therefore, we undertook to provide data for the peptides of interest to us.

EXPERIMENTAL

Pre-coated silica gel thin-layer sheets (J. T. Baker, Phillipsburgh, N.J., U.S.A.) were used. Amino acid and peptide standards were commercial materials obtained from Nutritional Biochemicals (Cleveland, Ohio, U.S.A.). DNP derivatives were made in the usual manner[3]. 1 μl of the DNP peptide (10^{-2} *M*, acetone) was applied 3 cm from the bottom of the plate and allowed to run 17 cm. The solvents were those recommended by Brenner *et al.*[3]: (A) benzene–pyridine–acetic acid (80:20:2) and (B) chloroform–methanol–acetic acid (95:5:1).

RESULTS AND DISCUSSION

From Table I it can be seen that oligopeptides of glycine separate well and allow unambiguous identification of the corresponding DNP peptide. For the mixed dipeptides there is not very good separation between glycylalanine and alanylglycine, but the other pairs separate well. Using this method in conjunction with the amino acid analyzer, it should be possible to identify the oligopeptides and mixed dipeptides of glycine, valine and alanine.

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TABLE I

$R_F \times 100$ VALUES FOR DNP DERIVATIVES

Derivative	*Solvent*	
	A	*B*
Glycine	14	43
Glycylglycine	5	20
Glycylglycylglycine	2	3
Glycylglycylglycylglycine	0	0
Valine	20	43
Valylvaline	21	39
Alanine	7	59
Alanylalanine	4	39
Alanylglycine	2	27
Glycylalanine	2	32
Glycylvaline	31	31
Valylglycine	20	27

ACKNOWLEDGEMENTS

We thank the National Research Council of Canada for the award of a post-doctoral Fellowship (to D.J.P.) and Professor B. Belleau for providing laboratory space.

REFERENCES

1 J. G. Heathcote, R. J. Washington, B. J. Keogh and R. W. Glanville, *J. Chromatogr.*, 65 (1972) 397.
2 D. W. Russell, *Biochem. J.*, 87 (1963) 1.
3 M. Brenner, A. Niederwieser and G. Pataki, in E. Stahl (Editor), *Thin-Layer Chromatography*, Springer, New York, 1969, pp. 756–772.