Journal of Chromatography, 80 (1973) 144–145
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 6651

Note

Thin-layer chromatographic separation of dipeptide and tripeptide diastereoisomers

PATRICK HUBERT and EDITH DELLACHERIE

Laboratoire de Chimie-Physique Macromoleculaire, C.N.R.S. E.R.A. No. 23, 1 rue Grandville, 54000-Nancy (France)

(Received January 11th, 1973)

Of the various methods that have been proposed for testing the optical homogeneity of synthetic peptides, chromatography has been used to the greatest extent.

Gas chromatography[1] often requires the preparation of suitably modified derivatives, which may present some difficulties.

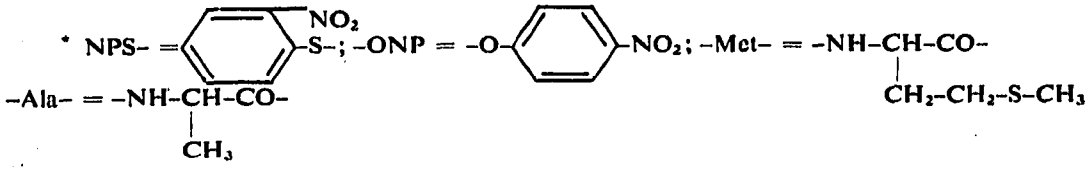
The chromatographic separation of diastereoisomeric peptides has also been studied on Sephadex[2], ion-exchange resins[3,4] and paper or thin layers (for reviews, see ref. 5). Using this last procedure, Taschner *et al.*[6], Wieland and Bende[2] and Pravda *et al.*[7] obtained successful results with dipeptide diastereoisomers, either as the free peptides or the N-protected methyl esters. Only a few studies on the chromatographic separation of diastereoisomeric tripeptides have been described in the literature[8].

In this paper, we report the results of the thin-layer chromatographic separation of diastereoisomeric *p*-nitrophenyl esters of N-protected di- and tripeptides.

EXPERIMENTAL

Starting from pure L-methionine and DL-alanine, we have synthesized the two oligopeptides NPS-LMet-DLAla-ONP* and NPS-LMet-LMet-DLAla-ONP* using the "backing-off" procedure of Goodman and Stueben[9], which is known to avoid epimerization.

The chromatographic separation of the diastereoisomers was performed on Silica Gel F_{254} (pre-coated plates, Merck Plastikfolien) with dry solvents (acetic acid–diethyl ether) to prevent the hydrolysis of the *p*-nitrophenyl esters. The detection was carried out with iodine.



RESULTS AND DISCUSSION

The R_F values obtained for the dipeptide (eluent: acetic acid–diethyl ether, 1.5:20, v/v) and for the tripeptide (eluent: acetic acid–diethyl ether 0.2:20, v/v) are shown in Table I.

TABLE I

R_F VALUES FOR THE DIPEPTIDE AND TRIPEPTIDE

Property	*NPS-Met-Ala-ONP*		*NPS-Met-Met-Ala-ONP*	
	L–L	L–D	L–L–L	L–L–D
M.p.(°C)	155–156	150	163–164	165–166
[α]	$[\alpha]^{23°}_{589\,nm} = -68.6°$*	$[\alpha]^{23°}_{589\,nm} = -52.1°$*	$[\alpha]^{22°}_{546\,nm} = -39.1°$**	$[\alpha]^{22°}_{546\,nm} = +36.2°$**
R_F	0.77	0.68	0.58	0.66

* concentration = 1 g/100 ml (acetonitrile).
** concentration = 0.6 g/100ml (dimethylformamide).

Taking these results as references, we have been able to control the optical homogeneity of the same activated peptides prepared by different methods and also to determine the extent of epimerization by comparing the optical rotation of the peptides studied with that of the pure diastereoisomers.

In the same way, the occurrence of epimerization during the following coupling reaction[10], in the presence of an amine, has been studied:

NPS–Met–Ala–ONP + HCl, H–Met–OMe $\xrightarrow{\text{Amine}}$ NPS–Met–Ala–Met–OMe

For this purpose, we separated the two epimers L–L–L and L–D–L corresponding to the tripeptide NPS–Met–Ala–Met–OMe on Silica Gel F_{254} (Merck Alurolle) with diethyl ether–isopropanol (20:0.25), and the following R_F values were obtained: L–L–L, $R_F = 0.56$; L–D–L, $R_F = 0.52$.

REFERENCES

1 F. Weygand, D. Hoffmann and A. Prox, *Z. Naturforsch.*, 23b (1968) 279.
2 T. Wieland and H. Bende, *Chem. Ber.*, 98 (1965) 504.
3 J. M. Manning and S. Moore, *J. Biol. Chem.*, 243 (1968) 5591.
4 N. Izumiya, M. Muraoka and H. Aoyagi, *Bull. Chem. Soc. Jap.*, 44 (1971) 3391.
5 M. Bodanszky and M. A. Ondetti, *Peptide Synthesis*, Interscience, New York, 1966, p. 153.
6 E. Taschner, L. Lubiewska, M. Smulkowski and H. Wojciechowska, *Experientia*, 24 (1968) 521.
7 Z. Pravda, K. Podůska and K. Bláha, *Collect. Czech. Chem. Commun.*, 29 (1964) 2626.
8 H. Sachs and E. Brand, *J. Amer. Chem. Soc.*, 76 (1954) 1811.
9 M. Goodman and K. C. Stueben, *J. Amer. Chem. Soc.*, 81 (1959) 3980.
10 E. Dellacherie, P. Hubert and J. Neel, *C. R. Acad. Sci., Ser. C*, 274 (1972) 556.