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Normal- and reversed-phase high-performance liquid chromatography of some phosphonodipeptides

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ABSTRACT

The chromatographic behaviour of some phosphonodipeptides on octadecyl, nitrile and aminopropyl sorbents was studied. The retention and selectivity parameters of the separation of phosphonopeptide diastereoisomers on the sorbents were determined. The diastereoisomers of the unprotected phosphonopeptides can be separated at pH <5 by reversed-phase high-performance liquid chroamtography. The separation of the protected diastereoisomers can be obtained on nitrile and aminopropyl sorbents by use hexane–isopropanol eluents.

INTRODUCTION

Aminophosphonic acids and peptides have high biological activity [1,2]. It is important to obtain these compounds with high optical purity, but few studies have been devoted to the liquid chromatography of phosphonopeptides. The separation of diethyl esters of 1-(N-L-alanylamino)benzylphosphonic acid isomers by ion-exchange chromatography has been described [3]. The diastereoisomers of L-phenylalanyl-1-aminoalkylphosphonic acids dialkyl esters have been separated on silica, but not very succesfully [4].

The aim of this work was to study the possibilities of applying different modes of high-performance liquid chromatography (HPLC) for the separation of phosphonopeptide (PP) diastereoisomers that differ in the configuration of the α-carbon atom of the aminophosphonic acid residues.

EXPERIMENTAL

Chromatographic conditions

The experiments were performed on an LKB (Bromma, Sweden) liquid chromatographic system consisting of a Model 2150 HPLC pump, a Model 7410 injector, a Model 2140 rapid spectral detector at 225 nm and a Model 2200 recording integrator. The column used were as follows: (1) octadecyl polyol Si 100, 5 μm, 250 × 4.6 mm I.D., (Serva, Heidelberg, Germany); (2) Separon SIX C_{18}; (3) Separon SIX NH_2; and (4) Separon SIX CN (the last three all 5 μm, 150 × 3.3 mm I.D.) (Tessek,

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Prague, Czechoslovakia). The mobile phases were methanol–10^{-2} *M* phosphate buffer (0:100 to 60:40) (10^{-2} *M* H_3PO_4 adjusted with ammonia) for columns 1 and 2 and hexane–isopropanol (1:99–20:80) for columns 3 and 4. The flow-rate was 0.5 ml/min for column 1 and 0.25 ml/min for columns 2–4.

Materials

Phosphonopeptides were obtained as described [5,6]. Methanol, hexane, isopropanol, orthophosphoric acid and ammonia (analytical-reagent grade) were used as received. Water was doubly distilled and filtered for HPLC use.

RESULTS AND DISCUSSION

The least hydrophobic phosphonopeptide L-Ala-L,D-Ala P[a] (I) has a low retention on octadecyl sorbent. In this instance it is necessary to use eluents with high ionic strength. The retention and selectivity (α) of the separation of PP diastereomers increase with increasing concentration of ammonium sulphate in the mobile phase (MP) (Table I). For L-Val–L,D-Ala P (II) the selectivity of the separation of the diastereoisomers is 2.16 when the MP does not include methanol.

TABLE I

RETENTION OF L–D ISOMERS (k') AND SELECTIVITY OF SEPARATION OF PP DIASTEREOISOMERS

Peptide	Column	Methanol (%)	k'_2	$\alpha = k'_2/k'_1$
I	1	1.5[a]	1.469	2.17
		3.0[a]	3.384	5.76
II	1	0	0.735	2.16
		10	0.337	1.55
III	1	30	2.145	1.4
		40	1.02	1.43
	2	30	9.11	1.73
		40	4.72	1.65
VI	1	62	4.26	1.17

[a] Concentration of $(NH_4)_2SO_4$ (*M*).

A further increase in the hydrophobicity of dipeptides [L-Leu–D,L-Phe P (III) and L-Phe–L,D-Leu P (IV)] does not result in an improved separation of diastereoisomers. A comparison of the chromatographic behaviour of dipeptide L-Leu–D,L-Phe (V) with its phosphonic analogues (III) shows that the latter is characterized, under comparable conditions, by both a smaller retention time and a smaller selectivity of separation of diastereoisomers (Fig. 1). Replacement of a carboxylic by a phosphonic group has a stronger effect on the retention of L,D-peptides.

[a] Abbreviations: we generally used accepted abbreviations for α-aminophosphonic acids with index P, *e.g.*, L,D-1-(N-L-alanylamino)ethylphosphonic acids (L-Ala–L,D-Ala P), diethyl ester of L,D-1-(N-benzyloxycarbonyl-L-phenylalanylamino)-3-methylbutylphosphonic acid [*Z*-L-Phe–L,D-Leu $P(OEt)_2$].

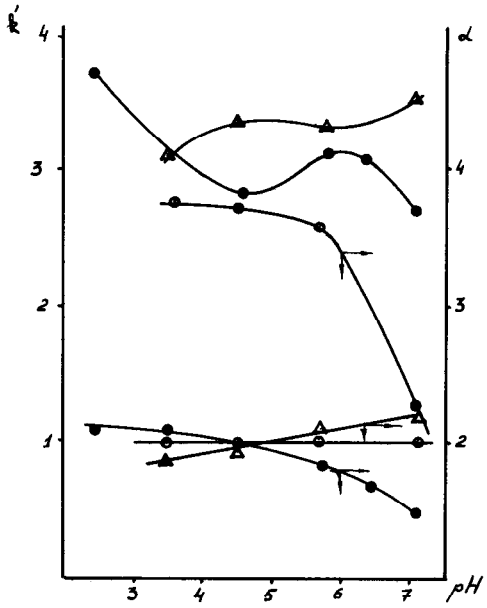

Fig. 1. Influence of pH on capacity factors (k') of L–L isomers and selectivity (α) of PP diastereoisomer separation by RP-HPLC. Compounds: ○ = II; ● = III; △ = V. For conditions, see Experimental.

The retention time and selectivity of separation of phosphonodipeptide diastereoisomers increase appreciably with decreasing pH in the range 5–7, which corresponds to one of the pK values for dissociation of a phosphonic group (pK ≈ 5–6) (Fig. 1). The amino acid sequence of isomeric PP [L-Leu–L,D-Phe P (III) and L-Phe–L,D-Leu P (IV) has a weak effect on the retention time of L,L-diastereoisomers, whereas the retention of L,D-diastereoisomers is affected more appreciably, resulting in different selectivities (Fig. 2).

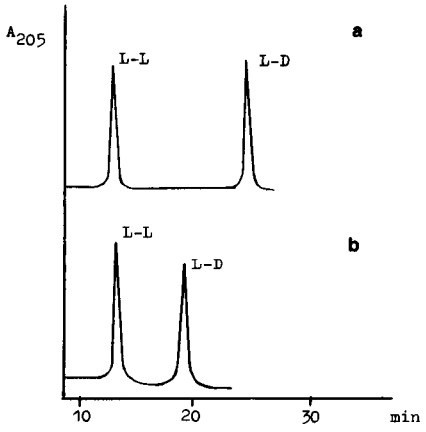

Fig. 2. Chromatograms of the separation of PP diastereoisomers by RP-HPLC. Column 1; eluent, methanol–0.01 M phosphate buffer (pH = 7.1) (40:60); flow-rate 0.5 ml/min. Compounds: (a) IV and (b) (III).

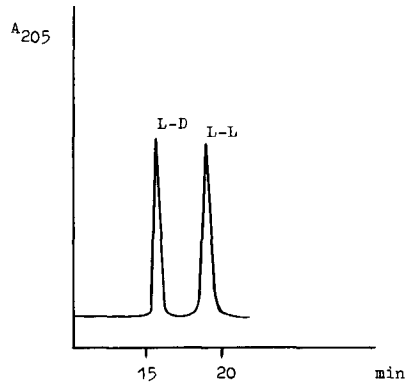

Fig. 3. Chromatogram of the separation of PP diastereoisomers (IX). Column 4: eluent, hexane–isopropanol (98:2); flow-rate, 1.0 ml/min.

Protected peptides are sorbed much more efficiently on octadecyl sorbents, but it is possible to separate diastereoisomers on these only for the (*Z*)-L-Phe–D,L-Leu $P(OEt_2)_2$ (VI). Clearly, in protected PP with a sorbent the interaction occurs primarily between hydrophobic protected groups, whereas in unprotected compounds the dominant contribution to the interaction comes from those parts of the molecules which differ in their configurations.

To eliminate or decrease the contribution from hydrophobic protected groups to the binding with a sorbent, we applied chromatography on nitrile and aminopropyl sorbents with hexane–isopropanol mixtures as MP to separate protected PP (Fig. 3).

Table II gives the parameters of the equation that describes the dependence of the retention of isomers on the concentration of isopropanol in the MP. We succeeded in separating the diastereoisomers of all the protected PP studied.

Under identical conditions [hexane–isopropnol (99:1)], the selectivity of sep-

TABLE II

COEFFICIENTS OF THE EQUATION $LNk' = A - BLNC$

Eluent: isopropanol [concentration C% (v/v), range 1–20%] in hexane.

Peptide	Column	L–D Isomers		L–L Isomers	
		A	*B*	*A*	*B*
VI	3	3.21	1.25	3.31	1.29
	4	2.88	1.44	2.88	1.44
VII	3	3.01	1.29	3.55	1.41
	4	2.16	1.31	2.76	1.50
VIII	3	2.11	1.11	2.43	1.19
	4	1.38	1.27	1.56	1.34
IX	3	3.63	1.30	3.92	1.40
	4	3.28	1.44	3.48	1.52

aration of diastereoisomers on the nitrile sorbent decreases in the order (*Z*)-L-Val–L,D-Ala P(OiPr)$_2$ (VII) > Boc-L-Leu–L,D-Phe P (OiPr)$_2$ (VIII) > (*Z*)-Ala–L,D-Ala P(OiPr)$_2$ (IX), and on the aminopropyl sorbent in the order VII > VIII > VI. Generally, the selectivity of separation and retention of protected phosphonic dipeptides is higher on the nitrile sorbent

REFERENCES

1 J. G. Allen, F. R. Atherton, M. J. Hall, C. H. Hassall, S. W. Holmes, R. W. Lambert, L. J. Nishet and P. S. Ringrose, *Nature (London)*, 272 (1978) 56–58.
2 P. Mastalerz, L. Kupczyk-Subotkowska, Z. S. Herman and G. Laskawiec, *Naturwissenschaften*, 69 (1982) 46–47.
3 Y. P. Belov, V. A. Davankov, V. A. Cyrjapkin and C. V. Rogozyn, *Izv. Akad. Nauk U.S.S.R., Ser. Kim.*, 7 (1975) 1619–1620.
4 L. Kupczyk-Subotkowska and P. Mastalerz, *Int. J. Pept. Protein. Res.*, 21 (1983) 485–490.
5 F. R. Atherton, C. H. Hassal and R. W. Lambert, *J. Med. Chem.*, 29 (1986) 29–40.
6 V. A. Solodenko, T. H. Kasheva and V. P. Kukhar, *Zh. Obshch. Khim.*, 59 (1989) 2786–2787.