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Note

Chromatographic assignment of the configuration of 1-aminoalkanephosphonic acids

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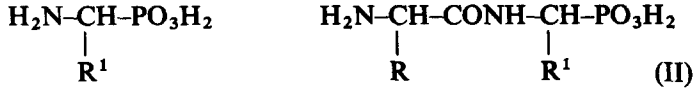
Aminophosphonic acids (I), analogues of naturally occurring amino acids* in which the carboxylic moiety is replaced by a phosphonic group, have became increasingly important compounds in recent years owing to their useful biological properties[1]. Unfortunately, nearly all the biological studies were carried out using racemic I, whereas the evaluation of their structure–activity relationships requires pure enantiomers of known configuration. Hence biological considerations provide a strong motivation for the synthesis and configurational assignment of optically active I.

Recently we described the separation of diastereomeric dipeptides containing P-terminal 1-aminoalkanephosphonic acids (phosphonodipeptides, II) by means of ion-exchange column chromatography and proposed the tentative assignments of their configurations based on the relative mobilities of diastereomers in thin-layer chromatography (TLC)[2,3].



(I) (II)

In this paper we report more extensive studies on the use of relative mobilities of phosphonodipeptides for the assignment of the configuration of aminophosphonates. We also describe the preparation of enantiomeric 1-aminoalkanephosphonic acids I (including unknown analogues of glutamic acid, adipic acid and proline) by acid hydrolysis of diastereomeric dipeptides II.

EXPERIMENTAL

Materials

The phosphonodipeptides were prepared as described earlier[3]. Their physicochemical data are given in Table I.

* Abbreviations: the standard abbreviations for protein amino acids are used. The phosphonic analogues of amino acids are abbreviated by the addition of P after the standard amino acid abbreviation (*e.g.*, AlaP for the phosphonic analogue of alanine). APP = 1-Aminopropanephosphonic acid.

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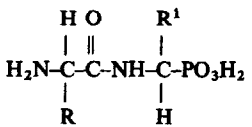
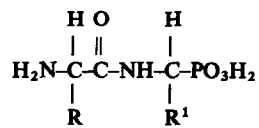
TABLE I

PHOSPHONODIPEPTIDES

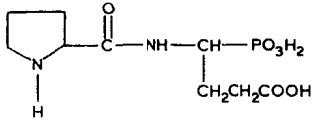
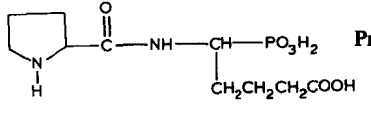
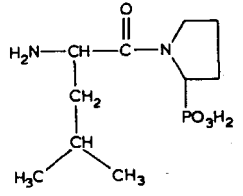


S,S(L,D)-Dipeptide



S,R(L,L)-Dipeptide

R	R^1	*Abbreviation*	*Mixture of (S,S)- and (S,R)-dipeptides*			*(S,S)-Dipeptide*		*(S,R)-Dipeptide*	
			Yield (%)	*M.p. (°C) dec.*	$[\alpha]_{578}^{20}$ (°) *(c 1,H_2O)*	*M.p. (°C) dec.*	$[\alpha]_{578}^{20}$ (°) *(c 1,H_2O)*	*M.p. (°C)*	$[\alpha]_{578}^{20}$ (°) *(c 1,H_2O)*
$(CH_3)_2CH$	CH_3	ValAlaP	72	251–257	+38 ± 1	263–265	+84 ± 1	278–279	− 9 ± 1
$(CH_3)_2CHCH_2$	CH_2CH_3	LeuAPP	65	254–256	+25 ± 1	268–269	+83 ± 1	255–256	−22 ± 1
CH_3	$(CH_3)_2CH$	AlaValP	74	262–264	+12 ± 1	266–268	+59 ± 1	278–281	−24 ± 1
$(CH_3)_2CH$	$(CH_3)_2CHCH_2$	ValLeuP	82	262–266	+30 ± 1	277–278	+87 ± 1	268–269	−23 ± 1
$(CH_3)_2CH$	CH_2CH_2COOH	ValGluP	23	249–253	−31 ± 1**	–	–	273–274	−34 ± 1
$(CH_3)_2CHCH_2$	CH_2CH_2COOH	LeuGluP*	61	223–224	+28 ± 1	196–198	+51 ± 1	237–239	−14 ± 1

		ProGluP	45	222–224	−29 ± 1***	199–202	−29 ± 1	–	–
$(CH_3)_2CH$	$CH_2CH_2CH_2COOH$	ValAdiP	39	247–252	+49 ± 1***	253–255	+49 ± 1	–	–
		ProAdiP	37	245–249	−44 ± 1***	–	–	246–268	−44 ± 1
		LeuProP*	30	266–268	+27 ± 1	268–269	+83 ± 1	255–256	−22 ± 1

* Separated by crystallization.
** Synthesis afforded more (*S,R*)-dipeptide.
*** Synthesis afforded more (*S,S*)-dipeptide.

Methods

TLC was performed on HP-TLC aluminium sheets pre-coated with silica gel $60F_{254}$ for nano-TLC (20 × 20 cm) (Merck) and plastic sheets pre-coated with cellulose F_{254} with a layer thickness of 0.1 mm (20 × 20 cm) (Merck). The chromatograms were developed using *n*-butanol-acetic acid-water (12:3:5) as the solvent and the run took 5.5 h for full development. The spots were rendered visible with ninhydrin spray reagent.

IR spectra were recorded on a Perkin-Elmer 621 instrument and NMR spectra on a Tesla BS 467 instrument at MHz. Although they are not given here, these spectra and the elemental analyses that were carried out on all the compounds described were consistent with their structures.

A glass column of 200 × 25 mm I.D. was used for the separation of diastereomeric phosphonodipeptides and also for the separation of 1-aminoalkanephosphonic acids from the mixtures obtained after hydrolysis of these peptides.

Procedure

Separation of diastereomers: a typical example. A 1.00-g amount of N-(*S*)-leucyl-(*SR*)-1-aminopropanephosphonic acid (L-Leu-DL-APP) was chromatographed on Dowex 50W-C8 (H^+) resin (50–100 mesh) with water as the eluent. The elution was monitored by the ninhydrin reaction. Fractions of 16 ml were collected. The (*S,S*)-dipeptide was obtained from fractions 175–265 (1440 ml) and weighed 400 mg. Fractions 266–330 yielded a mixture of diastereomers (230 mg), and (*S,R*)-phosphonodipeptide was collected in fractions 331–430 (1600 ml) and weighed 360 mg.

Separation of (S)-leucyl-(SR)-pyrrolidine-1-phosphonic acid (L-Leu-DL-ProP) by crystallization. A 1.30-g amount of (*S*)-leucyl-(*SR*)-pyrrolidine-1-phosphonic acid (L-Leu-DL-ProP) was refluxed with 15 ml of methanol for 10 min. After cooling to room temperature, the crystals were collected and (*S,R*)-diastereomer obtained in this manner was purified by repeating this procedure twice. A 0.5-g amount of chromatographically pure isomer was obtained.

The (*S,S*)-dipeptide was precipitated from the first methanol solution by addition of 10 ml of acetone. It was purified by dissolution in methanol and precipitation with acetone. This procedure was repeated twice, yielding 0.6 g of chromatographically pure diastereomer.

In a similar manner (*S*)-leucyl-(*SR*)-4-amino-4-phosphonobutyric acid (L-Leu-LD-GluP) was separated into diastereomers.

Preparation of optically pure 1-aminoalkanephosphonic acids: a typical example. A 450-mg amount of (*S*)-leucyl-(*R*)-pyrrolidine-1-phosphonic acid (L-Leu-L-ProP) was dissolved in 30 ml of concentrated hydrochloric acid and refluxed for 20 h. Then the solvent was evaporated in a rotary evaporator and the residue dissolved in small amount of distilled water, the impurities were filtered off and the products of hydrolysis were chromatographed on a Dowex 50W-X8 (H^+) (100–200 mesh) column. The elution was monitored using the ninhydrin reaction. Evaporation of the fraction containing L-pyrrolidine-1-phosphonic acid (as checked by TLC) yielded 200 mg (80% yield) of L-ProP.

RESULTS AND DISCUSSION

Diastereomeric mixtures of phosphonodipeptides II were synthesized starting from diethyl 1-aminoalkanephosphonates and carbobenzoxyamino acids by the mixed carboxylic–carbonic anhydride procedure[3] (Table I).

Diastereomeric phosphonodipeptides were usually separated by ion-exchange column chromatography. Thus, passing the mixture of diastereomers through a Dowex 50W-X8 column and simple elution with water resulted in separation of the isomers (Table I). This procedure, although time consuming, is the method of choice because of its simplicity and the high purity of the stereoisomers obtained.

We also achieved successful separations of diastereomeric LeuGluP and LeuProP by crystallization. With ValGluP, ProGluP, ValAdiP and ProAdiP the synthesis afforded nearly pure diastereomers, which were further purified by passage through a Dowex 50W-X8 (H^+) column.

In previous papers[2,3] we proposed the use of the relative mobilities of phosphonodipeptides II in TLC and also on an ion-exchange column for tentative assignments of the configurations of 1-aminoalkanephosphonic acids. For classical peptides it is well established[4] that L,D(*S*,*R*)-dipeptides migrate faster on paper and in TLC than the corresponding L,L(*S*,*S*)-isomers. Examples given in our previous papers[2,3] and here show that this rule also applies to phosphonodipeptides II. Thus, L,D(*S*,*S*)-phosphonodipeptides migrate faster on TLC (Table II) and a cation-ex-

TABLE II

R_F VALUES OF DIASTEREOMERIC PHOSPHONODIPEPTIDES

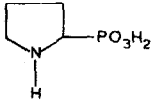
R_F values using *n*-butanol–acetic acid–water (12:3:5).

Peptide	R_F	
	Cellulose	*Silica gel*
(*S*)-Val-(*S*)-AlaP	0.60	0.15
(*S*)-Val-(*R*)-AlaP	0.46	0.10
(*S*)-Leu-(*S*)-APP	0.79	0.26
(*S*)-Leu-(*R*)-APP	0.67	0.20
(*S*)-Ala-(*S*)-ValP	0.52	0.28
(*S*)-Ala-(*R*)-ValP	0.45	0.21
(*S*)-Val-(*S*)-LeuP	0.92	0.48
(*S*)-Val-(*R*)-LeuP	0.83	0.41
(*S*)-Val-(*S*)-GluP	0.48	0.21
(*S*)-Val-(*R*)-GluP	0.30	0.15
(*S*)-Leu-(*S*)-GluP	0.54	0.27
(*S*)-Leu-(*R*)-GluP	0.45	0.21
(*S*)-Pro-(*S*)-GluP	0.19	0.08
(*S*)-Pro-(*R*)-GluP	0.24	0.12
(*S*)-Val-(*S*)-AdiP	0.42	0.26
(*S*)-Val-(*R*)-AdiP	0.36	0.20
(*S*)-Pro-(*S*)-AdiP	0.23	—*
(*S*)-Pro-(*R*)-AdiP	0.29	0.11
(*S*)-Leu-(*S*)-ProP	0.86	0.43
(*S*)-Leu-(*R*)-ProP	0.78	0.37

* Non-equimolar mixture of (*R*)- and (*S*)-isomers gave one spot with R_F = 0.09.

TABLE III

OPTICALLY ACTIVE 1-AMINOALKANEPHOSPHONIC ACIDS

R^1	*Abbreviation*	*Configuration of aminophosphonate*	*Yield (%)*	*M.p. (°C) dec.*	$[\alpha]_{578}^{20}$ (°) *(c 1, 1 M NaOH)*	$[\alpha]_{578}^{20}$ (°) *(c 1, 1 M NaOH) (from literature)*	*Ref.*
CH_3	AlaP	(*S*)-	78	263–265	+17 ± 1	+16,8	5
		(*R*)-	69	279–280	−17 ± 1	−16.9	5
CH_3CH_2	APP	(*S*)-	79	273–274	+21 ± 1	–	
		(*R*)-	90	274–275	−22 ± 1	–	
$(CH_3)_2CH$	ValP	(*S*)-	92	277–278	−0.8 ± 0.2*	−0.6	6
		(*R*)-	87	272–273	+0.8 ± 0.2*	+0.6	6
$(CH_3)_2CHCH_2$	LeuP	(*S*)-	84	278–281	+27 ± 1	+25	6
		(*R*)-	89	288–291	−28 ± 1	−24	6
CH_2CH_2COOH	GluP	(*S*)-	75**, 77***	179–282	+21 ± 1	–	
		(*R*)-	83**, 75§	183–184	−20 ± 1	–	
$CH_2CH_2CH_2COOH$	AdiP	(*S*)-	80	180–183	+13 ± 1	–	
		(*R*)-	81	192–194	−12 ± 1	–	
	ProP	(*S*)-	70	275–276	−60 ± 1	–	
		(*R*)-	80	272–273	+64 ± 1	–	

* (*c* 5, 1 *M* NaOH).
** From LeuGluP.
*** From (*S*)-Pro-(*S*)-GluP.
§ From (*S*)-Val-(*R*)-GluP.

change column than L,L(*S*,*R*)-isomers (the change in the Cahn–Ingold–Prelog notation results from the different priorities of phosphonate and carboxylic functions).

A reversal of relative mobilities occurs when L-proline is used as the N-terminal amino acid in the phosphonopeptide. This is in agreement with observatiosns for classical peptides[4], where the presence of N-terminal L-proline in a dipepotide causes faster migration of L,L- than L,D-isomers.

The validity of the relative mobility rule for phosphonodipeptides was established using information on both the literature data on the configuration of diastereomeric peptides (peptides of AlaP and APP; see refs. 2 and 3 and the literature cited therein) and the hydrolysis of diastereomeric phosphonopeptides to known enantiomeric 1-aminoalkanephosphonic acids I (AlaP, ValP, LeuP; see Table III).

Configurations of the obtained enantiomers of phosphonic analogues of glutamic acid (GluP), 2-aminoadipic acid (AdiP) and proline (ProP) and of enantiomeric 1-aminopropanephosphonic acid (APP) were assigned on the basis of the relative mobilities of their peptides (Table III).

There are only a limited number of enantiomeric 1-aminoalkanephosphonic acids described so far. Known examples include phosphonic analogues of alanine[5,6], valine[6,7], leucine[6], phenylalanine[6,8–10], tyrosine[9,11], serine[12], aspartic acid[13] and phenylglycine[8,14,15]. In this work we have added to this list the analogues of glutamic acid (GluP), proline (ProP), 2-aminoadipic acid (AdiP) and 1-aminopropanephosphonic acid (APP). Thus, hydrolysis of diastereomeric phosphonodipeptides, obtained by ion-exchange column chromatography, seems to be useful for the preparation of optically pure 1-aminoalkanephosphonic acids, which are desirable for biological purposes.

ACKNOWLEDGEMENT

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