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DERIVATIVES OF p-AMINOPHENYL-\beta-ALANINE AND
OF p-AMINOPHENYL-\alpha-ALANINE AND ITS DIPEPTIDES
THAT CONTAIN THE DIETHYLENIMIDOPHOSPHORYL GROUP
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In order to obtain less toxic and more active antitumor compounds we synthesized the ethyl ester of N_{β} -phthaloyl-N-(diethylenimidophosphoryl)-p-aminophenyl- β -alanine, and the derivatives of N-(diethylenimidophosphoryl)-p-aminophenyl- α -alanine and of the dipeptides, in which N-(diethylenimidophosphoryl)-p-aminophenyl- α -alanine is either the N-terminal (compounds of the A type) or the C-terminal (B type) amino acid.

 $\begin{pmatrix} \left| \begin{array}{c} N \right\rangle_{2} P(0)NH - \underbrace{-CHCH_{2}COOC_{2}H_{5}} \\ (I) NPhth \end{pmatrix}$ $R^{1} - \underbrace{-CH_{2}CHCOOR^{3}}_{R^{2}} R^{1} - \underbrace{-CH_{2}CHCONH - Val - OCH_{3}}_{R^{2}} \\ (II) - (VI) & (VII) - (IX) \\ R^{1} = NO_{2}, R^{2} = NPhth, R^{3} = CH_{3}(II), (VII); R^{1} = NH_{2}, R^{2} = NPhth, R^{3} = CH_{3}(III), (VIII) \\ R^{1} = NHP(O) \left(N \swarrow \right)_{2}, R^{2} = NPhth, R^{3} = CH_{2}(IV), (IX); R^{1} = NHP(O) \left(N \swarrow \right)_{2}, \\ R^{2} = NH^{2}, R^{3} = CH_{3}(V); R^{1} = NHP(O) \left(N \swarrow \right)_{2}, R^{2} = NH_{2}, R^{3} = Na(VI) \\ R^{4}NHCHCH_{2} - \underbrace{-}_{O} - NHP(O) \left(N \swarrow \right)_{2} \\ COOR^{2} \quad (X) - (XIX) \\ R^{1} = Phth - Ala, R^{2} = CH_{3}(X); R^{1} = Phth - Val, R^{2} = CH_{3}(XI); R^{1} = Ac - Ala, \\ R^{2} = CH_{3}(XII); R^{1} = Ac - Val, R^{2} = CH_{3}(XII); R^{1} = Ac - Ala, \\ R^{2} = CH_{3}(XV); R^{1} = Ac - Ala, R^{2} = Na(XII) \\ R^{1} = H - Ala, R^{2} = Na(XVII); R^{1} = Ac - Val, R^{2} = Na(XIV) \\ R^{1} = H - Ala, R^{2} = Na(XVII); R^{1} = H - Val, R^{2} = Na(XIX) \\ \end{array}$

The intermediate compounds for the indicated syntheses were obtained by the following methods. The esterification of N_{α} -phthaloyl-p-nitrophenylalanine gave the methyl ester of N_{α} -phthaloyl-p-nitrophenylalanine (II), the reduction of whose nitro group with hydrogen over Pd / CaCO₃ led to the methyl ester of N_{α} -phthaloyl-p-aminophenylalanine (III). The condensation of N_{α} -phthaloyl-p-nitrophenylalanine with the methyl ester of value in the presence of dicyclohexylcarbodiimide gave the methyl ester of N_{α} -phthaloyl-p-nitrophenylalanylvaline (VII), while the reduction of the latter by the above-described method led to the methyl ester of N_{α} -phthaloyl-p-aminophenylalanylvaline (VIII).

The diethylenimidophosphoryl group was inserted into the amino compounds in the usual manner. Thus, the long refluxing of the hydrochlorides of amines (III) and (VIII), and of the ethyl ester of N_{β}-phthaloyl-p-aminophenyl- β -alanine with excess POCl₃ led to the corresponding unstable N-dichlorophos-phoryl compounds, which, without isolation, were condensed with excess ethylenimine. In this way we obtained in satisfactory yield the ethyl ester of N_{β}-phthaloyl-N-(diethylenimidophosphoryl)-p-aminophenyl- β -alanine (I) and the methyl ester of N_{α}-phthaloyl-N-(diethylenimidophosphoryl)-p-aminophenylalanine (IV).

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- CH2CHCOR3	 4
\bigcirc	
R ¹ –	
TABLE	

		Å		1	6,81	9,0	1	1	5,59	
	ated, %	z	1	l	I	16,5	9,25	9,92	12,65	
	alcul	н	3,98	4,97	5,10	5,67	5,11	5,95	5,82	
		υ	61,02	66,67	58,15	45,70	60,91	65,24	58,57	
		Empirical formula	ClsH14N2O6	C ₁₈ H ₁₆ N ₂ O ₄	C22H23N405P	C13H18N4O3PNa †	C25H23N3O7	C23H25N205	C27H32N5O6P	
		<u>р</u> ,	1	l	6,76	8,20	ł	l	5,62	
	10	z		1	1	16, 35	9,27	10, 30	12, 30	
	Found	н	4,13	5,03	5,50	5,70	5,20	5,97	5,94	
		U	61,10	66,51	57,85	45,85	61, 14	65,74	i	
			107,5-109,5	130-132	156 - 157	230 *	152-154	179-181	171-173	
	Yield.	%	88	91	56	86	76	72	8,3	
			OCH ₃	OCH3	0CH3	ONa	NH-Val-00Ha	NII-Val-OCH3	NH-Val-OCH:	
R ²	31	:	NPhth	NPhth	NPhth	$\rm NH_2$	NPhth	NPhth	NPhth	
	13		NO2	NH2	a(>n)(0)4HN	NHP(O)(N<	NO2	211N	$NHP(O)(N\langle N \rangle)^{3}$	
	Com -	punod	(11)	(111)	(IV)	(VI)	(III)	(VIII)	(IX)	

*Decomposition temperature 50°. †Found: Na 6.10%, calculated: Na 6.70%.

TABLE 2. R^{1} NHCHCH₂ - \bigcirc - NHP(O)(N $\bigcirc)_{2}$ coor²

	1000														
- Punotato	Å		Yield,	Decomp.		F	% punc	_				Calo	oulated	η,	
nmoduro	-	4	%	temp., °C	υ	н	z	đ	Na	Empirical formula	U	Ħ	Z.	đ	Na
(X)	Phth-Ala	CH ₃	44	160	57,48	5,66	12,90	4,81		C25H28N5O6P	57,14	5,37	13,32	5,89	1
(IXI)	Phth-Val	CH3	06	100	58,58	5,94	12,87	5,53	1	C27H32N 506P	58,57	5,82	12,65	5,59	I
(XII)	Ac-Ala	CH ₃	36	120	1	6,39	۱	6,57	1	C19H28N6O5P		6, 45	1	7,08	l
(XIII)	Ac-Val	CH3	40	80	i	6,80	j	6,32	1	C21H32N6O6P	ł	6,93	1	6,65	- 1
(XIV)	H-Ala	CH3	60	80	51,90	6,62	l	7,26	i	C17H28N5O4P	51,62	6,63	1	7,83	l
(XV)	H-Val	CH ₃	80	100	54,04	7,18			Ì	C19H30N5O4P	53,89	7,14		.	İ
(XVI)	Ac-Ala	Na	93	70	44,95	5,98	l	6,42	4,77	C18H26N5O6PNa.2H20	44,91	6,07		6,43	4,78
(IIVX)	Ac-Val	Na	88	100	45,66	6,66		5,35	3,98	C20H29N6O6PNa.3H2O	45,54	6,69		5,87	4,36
(XVIII)	H-Ala	Na	6	235	46,58	5,88	1	7,07	4,92	C16H23N5O4PNa-0,5H2O	46,60	5,87	1	7,51	5,97
(XIX)	H-Val	Na	80	271	47,50	5,74	1	6,19	4,59	C18H27N5O4PNa·H2O	48,10	6,50	l	6,80	5,11

Removal of the phthaloyl group from (IV) by hydrazinolysis converted it to the methyl ester of N-diethylenimidophosphoryl)-p-aminophenylalanine (V),* the alkaline hydrolysis of which gave the corresponding water-soluble Na salt (VI). The methyl ester of N_{α} -phthaloyl-N-(diethylenimidophosphoryl)-p-aminophenylalanylvaline (IX) could not be obtained in satisfactory yield. It proved impossible to avoid the formation of a large amount of a polymer that was insoluble in water and in organic solvents, and whose structure was not established, by varying the reaction conditions and the methods of isolating the product. The constants of compounds (I)-(IX) are given in Table 1.

To obtain dipeptides of the B type the methyl ester of N-(diethylenimidophosphoryl)-p-aminophenylalanine (V) was condensed with the N_{α} -phthaloyl and N_{α} -acetyl derivatives of alanine and value by the dicyclohexylcarbodiimide method. In this way we obtained in good yields the methyl esters of N_{α} -phthaloylalanyl-, N_{α} -phthaloylvalyl-, N_{α} -acetylalanyl-, and N_{α} -acetylvalyl-N-(diethylenimidophosphoryl)-paminophenylalanine (X)-(XIII), and it was shown that the diethylenimidophosphoryl group is stable under the conditions of preparing dipeptides by the dicyclohexylcarbodiimide method. Removal of the phthaloyl group from dipeptides (X) and (XI) by hydrazinolysis converted them to the corresponding dipeptides (XIV) and (XV) with a free amino group. The alkaline hydrolysis of the methyl esters of the N_{α} -acetyl-protected dipeptides (XII) and (XIII), and of dipeptides (XIV) and (XV) with a free amino group, gave the corresponding water-soluble Na salts (XVI)-(XIX). The constants of compounds (X)-(XIX) are given in Table 2.

EXPERIMENTAL METHOD

<u>Methyl Ester of N_{$\alpha}-Phthaloyl-p-nitrophenylalanine (II)</u>. With cooling, a suspension of 23.44 g of N_{<math>\alpha$}-phthaloyl-p-nitrophenylalanine in 400 ml of abs. methanol was saturated with HCl for 8 h and allowed to stand overnight. Then the mixture was refluxed in an HCl stream for 3 h. The methanol was evaporated in vacuo to incipient crystallization, and then cooled. The obtained precipitate was filtered, washed well with water, and recrystallized from methanol. We obtained 21 g of methyl ester (II).</u></sub>

<u>Methyl Ester of N_Q-Phthaloyl-p-aminophenylalanine (III)</u>. A suspension of 17.7 g of nitro product (II) in 300 ml of methanol and 10 ml of water was stirred at 40-50° in the presence of 0.5 g of Pd/CaCO₃ in a H₂ atmosphere. After the calculated amount of H₂ had been absorbed (3.4 liters) the catalyst was filtered. The filtrate was evaporated in vacuo to incipient crystallization, and then cooled. We obtained 14.8 g of methyl ester (III) by filtration, which was recrystallized from methanol. A solution of 16.2 g of methyl ester (III) in 150 ml of abs. CH_2Cl_2 was saturated at 0° with HCl for 1 h. Precipitation with ether gave the hydrochloride of (III) with mp 199-203°.

In a similar manner we obtained the methyl ester of N_{α} -phthaloyl-p-aminophenylalanylvaline (VIII) (recrystallized from a water – MeOH mixture) and the hydrochloride of (VIII) with mp 150° (decomp.).

Methyl Ester of N_{α} -Phthaloyl-p-nitrophenylalanylvaline (VII). With stirring, a suspension of 79.1 g of N_{α} -phthaloyl-p-nitrophenylalanine in 580 ml of abs. CHCl₃ was added at 0° to a mixture of 50 g of N,N'-dicyclohexylcarbodiimide and 30.5 g of methyl DL-valinate in 230 ml of abs. CHCl₃. The reaction mixture was stirred for another 30 min and then let stand overnight at ~20°, after which it was cooled. We filtered off 46.1 g of N,N'-dicyclohexylurea, and the filtrate was evaporated in vacuo. The residue was dissolved in boiling ether, cooled, and filtered to give 80.16 g of (VII), which was recrystallized from an EtOAc -petroleum ether mixture.

Methyl Ester of N_{α} -Phthaloyl-N-(diethylenimidophosphoryl)-p-aminophenylalanine (IV). A suspension of 26.9 g of the hydrochloride of the methyl ester of N_{α} -phthaloyl-p-aminophenylalanine (III) (dried in vacuo over KOH) in 200 ml of freshly distilled POCl₃ was refluxed in a nitrogen atmosphere for 1 h. The excess POCl₃ was vacuum-distilled in a nitrogen atmosphere. The residue was washed with benzene and chilled ether. We obtained 21.1 g of the methyl ester of N_{α} -phthalolyl-N-(dichlorophosphoryl)-p-aminophenylalanine with mp 129-130°, which was dissolved in 200 ml of abs. CH₂Cl₂. With stirring, the obtained solution was added at -8° in a nitrogen atmosphere to a solution of 10 ml of ethylenimine in 80 ml of abs. CH₂Cl₂. The mixture was stirred for another 4 h at ~20°. Then 80 ml of benzene was added, the obtained β -chloroethylamine hydrochloride was filtered, and the filtrate was washed with water, dried over MgSO₄, and evaporated in vacuo. The syrupy residue was rubbed in abs. EtOAc and then washed with ether. We obtained 19.3 g of phosphoramide (IV), which was recrystallized from EtOAc.

The methyl ester of phosphoramide (IX) was obtained in a similar manner. From the hydrochloride of the ethyl ester of N_β -phthaloyl-p-aminophenyl- β -alanine, obtained as described in [1], was synthesized phosphoramide (I) in 24.4% yield, decomp. temp. 99°. Found: C 58.91; H 5.56; P 6.3%. C₂₃H₂₅N₄O₅P. Calc.: C 58.98; H 5.38; P 6.6%.

*The compound was not isolated analytically pure. 390 <u>Methyl. Ester of N-(Diethylenimidophosphoryl)-p-aminophenylalanine (V)</u>. With stirring, to a suspension of 2.27 g of the methyl ester of N_{α} -phthaloyl-N-(diethylenimidophosphoryl)-p-aminophenylalanine (IV) in 10 ml of abs. methanol was added 0.26 g of hydrazine hydrate in 10 ml of methanol. The mixture was stirred for 7 h, and then let stand at ~20° for 4 days. We filtered off 0.8 g of phthalylhydrazine, and the filtrate was evaporated in vacuo to give 1.45 g (90%) of methyl ester (V) as a colorless syrup.

The methyl esters of alanyl- and valyl-N-(diethylenimidophosphoryl)-p-aminophenylalanine (XIV) and (XV) were obtained in a similar manner. They were, respectively, recrystallized from acetone and an acetone – ether mixture (see Table 2).

<u>Methyl</u> Ester of N_{α} -Phthaloylalanyl-N-(diethylenimidophosphoryl)-p-aminophenylalanine (X). With stirring, a solution of 5.7 g of N_{α} -phthaloylalanine in 100 ml of abs. CHCl₃ was added at 0° to a mixture of 8.5 g of the methyl ester of N-(diethylenimidophosphoryl)-p-aminophenylalanine (V) in 40 ml of CHCl₃ and 5.42 g of dicyclohexylcarbodiimide in 40 ml of CHCl₃. The reaction mixture was stirred for 30 min, and then let stand overnight at ~20°. We filtered off 4.4 g of N,N'-dicyclohexylurea, the filtrate was evaporated in vacuo, and the residue was rubbed in acetone. We obtained 5.9 g of dipeptide (X), which was recrystallized from acetone.

Methyl esters (XI)-(XIII) were obtained in a similar manner. They were recrystallized as follows: (XI) from a 1:1 ether – acetone mixture, and (XII) and (XIII) from EtOAc.

Sodium Salt of N-(Diethylenimidophosphoryl)-p-aminophenylalanine (VI). To a solution of 1.45 g of the methyl ester of N-(diethylenimidophosphoryl)-p-aminophenylalanine (V) in 25 ml of methanol were added 4.3 ml of 1 N MeONa solution in methanol and 0.1 ml of water, and the mixture was let stand for 4 days. The solvent was evaporated in vacuo. The residue was dried by the azeotropic distillation of the water with benzene, and then rubbed in boiling acetone. Filtration gave 1.28 g of (VI), which was reprecipitated from methanol solution with ether.

The Na salts of N-acetylalanyl-(XVI), N-acetylvalyl-(XVII), alanyl-(XVIII), and valyl-N-(diethylenimidophosphoryl)-p-aminophenylalanine (XIX) were obtained in a similar manner.

CONCLUSIONS

1. Some N-diethylenimidophosphoryl derivatives of p-aminophenyl- α -alanine and p-aminophenyl- β -alanine were synthesized.

2. Some dipeptides were obtained, in which N-(diethylenimidophosphoryl)-p-aminophenylalanine is either the N- or C-terminal amino acid.

LITERATURE CITED

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