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Abstract

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Diethyleneimidophosphoryl and Diethyleneimidothiophosphoryl Derivatives of Amino Acids and Peptides

At the present time, a large amount of experimental material has accumulated which attests to the high antitumor activity of a group of synthetic preparations containing ethyleneimine rings: 2,4,6-triethyleneimino-*S*-triazine (TET) ([1,2]), triethyleneimide of phosphoric acid (TEF) ([3]), triethyleneimide of thiophosphoric acid (thioTEF) ([4–6]), *N*-benzyl-*N*′,*N*″,*N*‴,*N* -diethylenetriamide of phosphoric acid ([7]), 1,4-dioxyphenyl-*O,O*-bisdiethylenediamide of phosphoric acid ([8,9]), 2,5-dipropoxy-3,6-diethyleneiminobenzoquinone (E-39) ([10–12]), 2,5-diacetamido-3,6-diethyleneimino-1,4-benzoquinone ([13,14]), and others. Some derivatives of phosphoric acid have found the greatest practical application; among them thioTEF is widely used in the clinic. The use of these preparations in therapeutic practice could be more effective if they were introduced into the organism in large shock doses; however, this is limited by the strong side effect of the preparations on the bone marrow, which makes them of low effectiveness. It was shown earlier that the introduction of a di-(2-chloroethyl)amino group into amino acids and peptides leads to highly active and low-toxicity antitumor compounds ([15–22]). On the basis of this observation it should have been concluded that the introduction of diethyleneimidophosphoryl and thiophosphoryl groups into derivatives of amino acids and peptides should also lead to active antitumor compounds with a higher selectivity of action than that of the simplest ethyleneimidophosphoryl derivatives. The synthesis of this type of compounds was carried out by us through the interaction of an ester of an amino acid or peptide with phosphorus oxychloride, followed by replacement of the chlorine atoms in the intermediate dichloroanhydride (I) by an ethyleneimine residue.

$$\mathrm{H_2N{-}\langle\!\langle C_6H_4\rangle\!\rangle{-}CH_2R} \xrightarrow[\mathrm{N(C_2H_5)_3}]{\mathrm{POCl_3}} \mathrm{Cl_2P(=O){-}NH{-}\langle\!\langle C_6H_4\rangle\!\rangle{-}CH_2R} \xrightarrow[\mathrm{N(C_2H_5)_3}]{\text{ethyleneimine}}$$

(I)

$$\longrightarrow \mathrm{>N{-}P(=O){-}NH{-}\langle\!\langle C_6H_4\rangle\!\rangle{-}CH_2R} < \mathrm{N}$$

(II)

By this method were obtained: ethyl ester of *p*-(N-diethyleneimidophosphoryl)-aminophenylacetic acid (II, $R = -COOC_2H_5$); ethyl ester of *p*-(N-diethyleneimidothiophosphoryl)-aminophenylacetic acid; ethyl ester of *p*-(N-diethyleneimidophosphoryl)-aminobenzylphosphinic acid (II, $R = -PO(OC_2H_5)_2$); ethyl ester of *p*-(N-diethyleneimidophosphoryl)-amino-*N*-acetyl-DL-phenylalanine (II, $R = -CH(NHCOCH_3)COOC_2H_5$);

ethyl ester of p-(N-diethyleneimidophosphoryl)aminophenacetyl-DL-phenylalanine (II, R = $-CONHCH(CH_2C_6H_5)COOC_2H_5$) and ethyl ester of p-(N-diethyleneimidophosphoryl)amino-N-acetyl-DL-phenylalanyl-DL-valine (II, R = $-CH(NHCOCH_3)CONH-CH(COOC_2H_5)CH(CH_3)_2$). Biological tests of the compounds obtained will be reported later.

Experimental Part

Ethyl ester of p-(N-diethyleneimidophosphoryl)aminophenylacetic acid (II, R = $-COOC_2H_5$). To 8.55 g (55.7 mmol) of $POCl_3$ in 180 ml of abs. ether, 20 g (111.4 mmol) of ethyl ester of p-aminophenylacetic acid in 300 ml of abs. ether was added dropwise with stirring at 0–5°. After addition was complete, the reaction mixture was stirred with cooling for 30 min and then at room temperature for 3.5 h; the precipitated hydrochloride of ethyl ester of p-aminophenylacetic acid was filtered off, and the ethereal solution was added dropwise with stirring at 5–8° to a mixture of 11.3 g (112 mmol) of triethylamine and 4.82 g (112 mmol) of ethyleneimine in 200 ml of abs. ether. The reaction mixture was stirred with cooling for 1 h and then for 2 h at room temperature and left overnight. The precipitated triethylamine hydrochloride was filtered off, and the solvent was distilled off in vacuo to a small volume. The residue was filtered off and recrystallized from ethyl acetate–petroleum ether. Ethyl ester of p-(N-diethyleneimidophosphoryl)aminophenylacetic acid was obtained as colorless crystals, m.p. 111–113°. Yield 5.7 g.

$C_{14}H_{20}N_3O_3P$. Found, %: C 54.17; H 6.57; N 14.12; P 9.97
Calculated, %: C 54.35; H 6.52; N 13.59; P 10.03

Ethyl ester of p-(N-diethyleneimidothiophosphoryl)aminophenylacetic acid. To 4.72 g (27.9 mmol) of $PSCl_3$ in 50 ml of abs. ether, a mixture of 5.0 g (27.9 mmol) of ethyl ester of p-aminophenylacetic acid and 2.82 g (27.9 mmol) of triethylamine in 100 ml of ether was added with stirring at 0–4°. After addition was complete, the reaction mixture was stirred for 1 h with cooling and for 3 h at room temperature. Triethylamine hydrochloride was filtered off, and the ethereal solution was evaporated. The residue was mixed with chloroform (100 ml), and with stirring and cooling to 6–8° was added to 2.4 g (56.0 mmol) of ethyleneimine and 5.62 g (56.0 mmol) of triethylamine in 50 ml of chloroform. Stirring was continued with cooling for 1 h, and

the mixture was left overnight. The chloroform solution was washed with water and dried over $MgSO_4$. After distillation of the chloroform, the residue was washed with a small amount of ether and filtered off. Ethyl ester of p-(N-diethyleneimidothiophosphoryl)aminophenylacetic acid was obtained as colorless crystals, m.p. 113–115° (from benzene). Yield 1.5 g.

$C_{14}H_{20}N_3O_2PS$. Found, %: C 51.73; H 6.22; N 12.90; P 9.35; S 9.90
Calculated, %: C 51.69; H 6.15; N 12.92; P 9.54; S 9.84

Ethyl ester of p-(N-diethyleneimidophosphoryl)amino-N-acetyl-DL-phenylalanine (II, R = $-CH(NHCOCH_3)COOC_2H_5$). To a solution of 0.99 g (6.47 mmol) of freshly distilled $POCl_3$ in 15 ml of $CHCl_3$, a mixture of 1.61 g (6.47 mmol) of ethyl ester of N-acetyl-p-amino-DL-phenylalanine[23] and 0.655 g (6.47 mmol) of triethylamine in 40 ml of chloroform was added dropwise with stirring and cooling with ice water. After addition was complete, the reaction mixture was stirred for 1 h with cooling and then for 3 h at room temperature. The solution was added dropwise with stirring to a mixture of 0.51 g (11.85 mmol) of ethyleneimine and 1.32 g (11.85 mmol) of triethylamine in 20 ml of chloroform at 6–8°. After addition was complete, the solution was left overnight. The chloroform was washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by reprecipitation from ethyl acetate with petroleum ether. Ethyl ester of p-(N-diethyleneimidophosphoryl)ami-

···of N-acetyl-DL-phenylalanine—colorless crystals, m.p. 181–183°. Yield 0.4 g.

$C_{17}H_{25}O_4N_4P$.	Found, %:	C 53.54; H 6.69; P 7.90; N 14.90
	Calculated, %:	C 53.68; H 6.57; P 8.15; N 14.73

By an analogous method, the diethyl ester of p-(N-diethylenimidophosphoryl)-aminobenzylphosphinic acid was obtained (II, R = $-PO(OC_2H_5)_2$), as colorless crystals, m.p. 160–162° (from ethyl acetate). Yield 10%.

$C_{15}H_{25}N_3O_4P_2$.	Found, %:	C 48.32; H 6.53; P 16.86
	Calculated, %:	C 48.25; H 6.70; P 16.62

Ethyl ester of p-(N-diethylenimidophosphoryl)-aminophenacetyl-DL-phenylalanine (II, R = $CONHCH(CH_2C_6H_5)COOC_2H_5$). To a solution of 18.1 g (0.1 mole) of p-nitrophenylacetic acid in chloroform were added successively 20.6 g (0.1 mole) of 1,3-dicyclohexylcarbodiimide and 19.3 g (0.1 mole) of the ethyl ester of DL-phenylalanine in chloroform. The mixture was shaken and left overnight at room temperature. The precipitated 1,3-dicyclohexylurea was filtered off. The chloroform solution was washed with 1*N* HCl, bicarbonate, and water. It was then dried over $MgSO_4$ and evaporated in vacuo. The residue consisted of colorless crystals with m.p. 146°. Weight 31.7 g. After recrystallization from ethanol, the m.p. of ethyl p-nitrophenacetyl-DL-phenylalanine rose to 148°.

$C_{19}H_{19}N_2O_5$.	Found, %:	C 63.99; H 5.77; N 8.02
	Calculated, %:	C 64.23; H 5.35; N 7.99

3.75 g of ethyl p-nitrophenacetyl-DL-phenylalanine was reduced with hydrogen over $Pd(OH)_2CaCO_3$ in ethanol. The resulting oily ethyl p-aminophenacetyl-DL-phenylalanine (3.45 g; 10.5 mmol) was dissolved in 25 ml of chloroform; to the solution were added 1.06 g (10.5 mmol) of triethylamine, and the resulting mixture was added dropwise with stirring to 1.6 g (10.5 mmol) of freshly distilled phosphorus oxychloride in 13 ml of chloroform. After completion of the addition, the reaction mixture was stirred for 1 hour under cooling and then left at room temperature for 3 hours. Thereafter, 2.12 g (2.1 mmol) of triethylamine and 0.9 g (2.1 mmol) of ethylenimine in 32 ml of chloroform were added dropwise with stirring to the mixture at 6–8°. The mixture was left at room temperature for 4 hours. The solution was thoroughly washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was crystallized from ethyl acetate. Ethyl ester of p-(N-diethylenimidophosphoryl)-aminophenacetyl-DL-phenylalanine—colorless crystals, m.p. 145–147°. Yield 58%.

$C_{23}H_{29}N_4O_4P$.	Found, %:	C 60.22; H 6.55; N 11.53; P 6.56
	Calculated, %:	C 60.53; H 6.36; N 12.28; P 6.8

Ethyl ester of p-(N-diethylenimidophosphoryl)-amino-N-acetyl-DL-phenylalanyl-DL-valine (II, R = $-CH(NHCOCH_3)CONH-CH(COOC_2H_5)CH(CH_3)_2$). A mixture of 1.6 g (6.35 mmol) of N-acetyl-p-nitro-DL-phenylalanine, 0.93 g (6.35 mmol) of ethyl ester of DL-valine, and 1.31 g (6.35 mmol) of 1,3-dicyclohexylcarbodiimide in 10 ml of chloroform was left overnight. The precipitated 1,3-dicyclohexylurea was filtered off. The chloroform mother liquor was washed with 1*N* HCl, $NaHCO_3$, and water, then dried over $MgSO_4$ and distilled off in vacuo. The obtained crystals of ethyl N-acetyl-p-nitro-DL-phenylalanyl-DL-valine, m.p. 172–175°, were reduced with hydrogen in the presence of $Pd(OH)_2CaCO_3$ in ethanol. Ethyl ester of N-acetyl-p-amino-DL-phenylalanyl-DL-valine was dissolved in 12 ml of CH_2Cl_2, added to···

to a solution of 0.465 g (4.6 mmol) of triethylamine. The resulting mixture was added dropwise with stirring to 0.705 g (4.6 mmol) of $POCl_3$ in 6 ml of CH_2Cl_2 at 0–5°. After completion of the addition, the reaction mixture was stirred for 1 hour with cooling and then for 3 hours at room temperature. The solvent was distilled off. The residue was dissolved in 20 ml of CH_2Cl_2 and added dropwise with stirring to a mixture of 0.93 g (9.2 mmol) of triethylamine and 0.41 g (9.2 mmol) of ethylenimine at 6–8°. The reaction mixture was left at room temperature for 4 hours. The chloroform solution was washed with water, dried over $MgSO_4$, and evaporated in vacuo. The residue was crystallized from ethyl acetate. Ethyl *p*-(N-diethylenimidophosphoryl)-amino-N-acetyl-DL-phenylalanyl-DL-valinate—colorless crystals (0.5 g), mp 180–183°.

$C_{22}H_{34}N_5O_5P$.	Found, %:	C 55.10;	H 7.18;	P 5.68
	Calculated, %:	C 55.11;	H 7.1;	P 6.48

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Note: Figure translations are in progress. See original paper for figures.