PHOSPHORUS-CONTAINING AMINOCARBOXYLIC ACIDS WITH AN o-XYLYLENE FRAGMENT. III*

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Phosphorus-containing aminocarboxylic acids (PAA) of the general formula

$$(HO)_2P(O)(CH_2)_nCH(NH_2)COOH \qquad (I)$$

represent a new and promising class of antispasmodic compounds, which have been studied extensively in recent years [1, 3-8]. 2-Amino-5-phosphonovaleric acid (I, n = 3) and 2-amino-7-phosphonoheptanoic (I, n = 5) acids exhibit the highest antispasmodic activity [1, 5-7] and are strong selective antagonists of N-methyl-D-aspartic (NMDA) receptors, which probably also determines their antispasmodic properties [4, 7, 8]. The hydrophilicity of the compounds probably does not promote the permeability of the molecule through the hematoencephalic barrier [7]. It can be assumed that the substitution of the hydrocarbon chain of the "canonical" molecules by a more lipophilic fragment will lead to an increase in the antispasmodic effect. However, the o-phenylene analogs of the most effective acid (I, n = 5) – the isomers

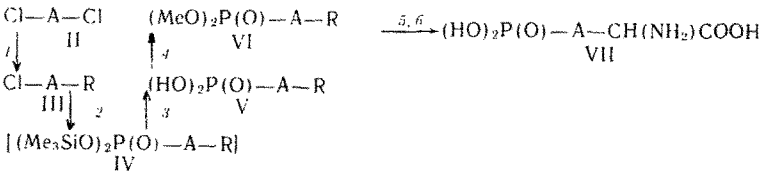
$$o\text{-}[(HO)_2P(O)CH_2]—C_6H_4—CH_2CH_2CH(NH_2)COOH$$
$$o\text{-}[(HO)_2P(O)CH_2CH_2]—C_6H_4—CH_2CH(NH_2)COOH$$

have a very low affinity to the NMDA receptor, whereas the p-phenylene analog of the inactive acid (I, n = 6) having the structure

$$n\text{-}[(HO)_2P(O)CH_2]—C_6H_4—CH_2CH(NH_2)COOH,$$

displays a high affinity to the above-mentioned receptor [4]. In this case, possibly, the geometrical factor is more important, i.e., the particular position of the functional groups in the PAA molecule, which correspond to the active centers of the NPDA receptor. Introduction of an aromatic fragment into the molecule of PAA probably reduces the distance between the phosphono and amino acid functions and increases the rigidity of the molecule. Therefore, the o-phenylene derivatives of acid I (n = 5) are more likely to be analogs of acid I (n = 4) which does not have antispasmodic properties [1, 5-7]. On this basis, the p-phenylene derivative of acid I (n = 6) can be regarded as an analog of acid I (n = 5), which is the most active and most comprehensively investigated compound in the PAA series [1, 5-7]. It was therefore of interest to synthesize the o-phenylene derivative of the inactive acid I (n = 4), which would possibly display the properties of the active compound I (n = 3), having weaker antispasmodic properties, but a higher affinity with respect to NMDA-receptor than acid I (n = 5) [1, 7].

The present work is devoted to the synthesis and investigation of the antispasmodic and antihypoxic properties of novel PAA, containing an o-xylylene fragment between the phosphoric and amino acid functions of the molecule, i.e., derivatives of acid I (n = 4).



*Preceding articles, see [1-3].

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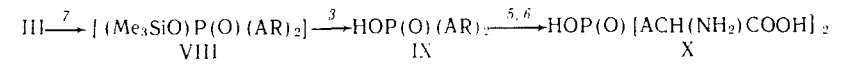

A: o-CH_2—C_6H_4—CH_2; R: C(NHAc)(COOEt)$_2$;
1 — NaC(NHAc)(COOEt)$_2$; *2* — {H_3PO_3 + $(Me_3Si)_2NH$};
3 — EtOH; *4* — $CH(OMe)_3$; *5* — HCl; *6* — OCH_2CHMe;
7 — {H_2POONH_4 + $(Me_3Si)_2NH$}

The alkylation of the sodium salt of the acetamidomalonic ester by an excess of o-xylylene dichloride (II) gave o-chloromethylbenzylacetamidomalonic ester (III) with a small admixture of a bisalkylation product. Phosphorylation of ester III by tris-(trimethylsilyl) phosphite, formed in the reaction mixture from phosphorous acid and hexamethyldisilazane, followed by alcoholysis of the bis(trimethylsilyl) ester (IV), leads to phosphonic acid (V). Treatment of V with an excess of trimethyl orthoformate gives the dimethyl ester (IV). Acid hydrolysis of acid V or ester VI results in the desired phosphonic-aminocarboxylic acid (VII).

Reaction of ester III with (trimethylsilyl) hypophosphite, formed in the reaction mixture from ammonium hypophosphite and hexamethyldisilazane [2], followed by the alcoholysis of the silyl ester (VIII) gave the phosphinic acid (IX), which contains two o-xylyleneacetamidomalonic fragments. Acid hydrolysis of compound IX leads to the phosphinic-bisamino acid (X).

EXPERIMENTAL (CHEMICAL)

The ^1H and ^{31}P NMR spectra were recorded on a Bruker CXP-200 Fourier-type spectrometer, using TMS (internal) and 85% H_3PO_4 (external) as standards; the weak-field shifts were considered to be positive. In taking the spectra of acids, the chemical shifts were determined with reference to the H_2O band (4.8 ppm). The melting points were measured on a Boetius–PHMK apparatus. For the column chromatography a silica gel L100-250μ was used. The elemental analysis data corresponded to the calculated values.

o-Chloromethylbenzylacetamidomalonic Ester (III). A 44.9 g portion (0.207 mole) of o-xylylene dichloride was added at 20°C to a solution of acetamidomalonic ester salt, obtained from 15.5 g (0.072 mole) of acetamidomalonic ester and 1.6 g (0072 mole) of Na in 40 ml of alcohol. The mixture was boiled with stirring for 5 h, NaCl was separated, and the filtrate was evaporated in vacuo. The residue was dissolved in 50 ml of $CHCl_3$, and the solution was washed (3 × 25 ml). The organic layer was dried over $MgSO_4$ and evaporated in vacuo. From the residue, 30.3 g of unreacted o-xylylene dichloride was distilled off under vacuum, bp 85-88°C (0.5 mm Hg). Chromatography of the residue on silica gel (eluent – benzene) gave 13.9 of ester III (55%), mp 78-80°C (hexane), R_f 0.80, ($CHCl_3$–acetone, 4:1). PMR spectrum ($CDCl_3$, δ, ppm); 1.24 t (6H, CH_3); 2.96 s [3H, $CH_3C(O)$]; 3.80 s (2H, CH_2CN); 4.24 q (4H, CH_2O); 4.59 s (CH_2Cl); 6.90 s (1H, NH); 7.03-7.40 m (4H, CH). $C_{17}H_{22}ClNO_5$.

A bisalkylation product, o-xylene-bis(acetamidomalonic) ester, is formed as an impurity and was isolated chromatographically in an amount of 2.7 g (7%), mp 150-152°C (alcohol–hexane), R_f 0.55 ($CHCl_3$–acetone, 4:1). PMR spectrum ($CDCl_3$, δ, ppm) 1.31 t (12H, CH_3); 2,04 s [6H, $CH_3C(O)$]; 3.60 s (4H, CH_2CN) 4.26 q (8H, CH_2O); 6.48 s (2H, NH); 6.90-7.20 m (4H, CH). $C_{26}H_{36}N_2O_{10}$.

o-(Dihydroxyphosphinylmethyl)benzylacetamidomalonic Ester (V). A mixture of 6.2 g (0.018 mole) of ester III, 1.6 g (0.020 mole) of H_3PO_3 and 8.1 ml (0.038 mole) of hexamethyldisilazane was boiled for 10 h with stirring (until the evolution of NH_3 ceased). After cooling, 20 ml of alcohol was added, the mixture was boiled for 15 min, evaporated in vacuo, and the residue was dissolved in 60 ml of $CHCl_3$. The solution was washed with water (2 × 10 ml). The organic layer was evaporated in vacuo, and the traces of water were removed by azeotropic distillation with benzene. The oily residue was identified by the ^1H and ^{31}P NMR spectroscopy methods as acid V, which was then used for the preparation of dimethyl ester VI and amino acid VII (see below). PMR spectrum ($CDCl_3$, δ, ppm): 1.28 t (6H, CH_3); 2.05 [3H, $CH_3C(O)$]; 3.03 d [2H, CH_2P, ($^2J_{PCH}$ 22.0 Hz)]; 3.75 s (2H, CH_2CN); 4.20 m (4H, CH_2O); 6.70 s (1H, NH); 6.80-7.30 m (4H, CH), ^{31}P NMR spectrum ($CDCl_3$, δ, ppm): 20.5, R_f 0.15 ($CHCl_3$–acetone, 4:1).

o-Dimethoxyphosphinylmethyl)benzylacetamidomalonic Ester (VI). A 19 ml portion (0.175 mole) of $HC(OMe)_3$ was added to acid V obtained from 6.2 g (0.018 mole) of ester III and 1.6 g

(0.020 mole) of H_3PO_3 in the preceding experiment, and the mixture was boiled for 2 h with stirring with distillation of MeOH. Chromatography of the residue on silica gel (eluent – $CHCl_3$) gave 4 g of VI (53%, based on ester III), mp 105-108°C (alcohol–hexane), R_f 0.30 ($CHCl_3$–acetone, 4:1). PMR spectrum ($CDCl_3$, δ, ppm): 1.28 t (6H, CH_3); 2.10 s [3h, $CH_3C(O)$]; 3.18 d [2H, CH_2P ($^2J_{PCH}$ 22.0 Hz)]; 3.64 d [6H, CH_3OP ($^3J_{POCH}$ 11.0 Hz)]; 3.76 s (2H, CH_2CN); 4.27 q (4H, CH_2O); 6.70 s (1H, NH); 7.00-7.40 m (4H, CH). ^{13}P NMR spectrum ($CDCl_3$, δ, ppm) 29.2. $C_{19}H_{29}NO_8P$.

β-[o-(Dihydroxyphosphinylmethyl)phenylalanine (VII). Method A. A mixture of 3 g (0.007 mole) of phosphonate VI and 12 ml of 8 N HCl was boiled for 12 h. The reaction mixture was extracted with ether (2 × 10 ml) and evaporated under vacuum. Hydrochloric acid was removed by repeated (3-4 times) addition of distilled water (10 ml portions), followed by its evaporation under vacuum. Traces of water were removed by azeotropic distillation with benzene. The residue was dissolved in 10 ml of alcohol and 1 ml of propylene oxide was added dropwise. The crystals that separated out were washed with alcohol, and dried in vacuo at 90°C. The yield of VII was 1.2 g (60%), mp 238-244°C (dec.). PMR spectrum (D_2O, δ, ppm): 3.02 d [2H, CH_2P ($^2J_{PCH}$ 16 Hz)]; 3.30 m (2H, CH_2C); 4.00 br. s (1H, CHN); 7.20 m (4H, CH). ^{31}P NMR spectrum (D_2O, δ, ppm) 20.8 (pH 5.0). $C_{10}H_{14}NO_5P \cdot H_2O$.

Method B. Acid V, obtained from 6.2 g (0.018 mole) of ester III, and 1.6 g (0.020 mmole) of H_3PO_3 were dissolved in 10 ml of water, and the mixture was extracted with ether (2 × 5 ml). A 20 ml portion of conc. HCl was added to the solution, the mixture was boiled for 16 h, extracted with $CHCl_3$ (2 × 10 ml) and evaporated under vacuum. The residue was dissolved in 15 ml of alcohol, and 2 ml of propylene oxide was added dropwise. The precipitate that separated out was filtered off, washed with alcohol and dried under vacuum at 90°C. The physical constants of the compound coincide with those given above. The yield of VII was 0.8 g (43%, based on ester III).

Bis[o-(acetamidodiethylmalonyl)benzyl]phosphinic Acid (IX). A mixture of 10.6 g (0.030 mole) of ester III, 1.4 g (0.016 mole) of ammonium hypophosphite and 6.9 ml (0.032 mole) hexamethyldisilazane was boiled for 9 h, with stirring, in an argon atmosphere. A 25 ml portion of alcohol and 2 ml of water were added dropwise to the reaction mixture at 20°C. The mixture was boiled for 30 min and evaporated under vacuum. The residue was dissolved in 40 ml of $CHCl_3$ and the solution was washed with water (3 × 10 ml). The organic layer was evaporated in vacuo. Chromatography of the residue on silica gel [eluent: $CHCl_3$, $CHCl_3$–i-PrOH, 10: (1-3)] gave 4.3 g (40%, based on ammonium hypophosphite) of phosphinic acid (IX), mp 165-167°C (alcohol–hexane). PMR spectrum ($CDCl_3$, δ, ppm) 1.29 g (12H, CH_3); 1.96 s [6H, $CH_3C(O)$]; 2.88 d [4H, CH_2P ($^2J_{PCH}$ 16 Hz)]; 3.45 br, s (4H, CH_2CN); 4.23 q (8H, CH_2O); 6.66 s (2H, NH); 6.90-7.20 m (8H, CH); 9.60 br. s (1H, POOH). ^{31}P NMR spectrum ($CDCl_3$, δ, ppm): 50.7. $C_{34}H_{45}N_2O_{12}P$.

Bis[o-(2-amino-2-carboxyethyl)benzyl]phosphinic Acid (X). A mixture of 4 g (0.006 mole) of phosphinic acid IX, 20 ml of 8 N HCl and 0.25 g of activated charcoal was boiled for 14 h. The charcoal was filtered off, the filtrate was extracted with ether (2 × 15 ml) and the extract was evaporated in vacuo. Hydrochloric acid was removed by three additions of distilled water (10 ml portions) with its subsequent evaporation under vacuum. The residue was dissolved in 15 ml of alcohol and 1 ml of propylene oxide was added to the solution. The precipitate that separated out was washed with alcohol and dried in vacuo at ∿130°C. Yield 1.5 g of X (63%), mp 265-268°C (dec.) PMR spectrum (D_2O, δ, ppm): 3.00 d [4H, CH_2P ($^2J_{PCH}$ 14 Hz]; 3.16 s (4H, CH_2, CN); 3.96 br. s (2H, CHN); 7.18 br. s (8H, CH). ^{31}P NMR spectrum (D_2O, δ, ppm): 47.7 (pH 1); 36.4 (pH 6). $C_{20}H_{25}N_2O_6P \cdot H_2O$.

EXPERIMENTAL (PHARMACOLOGICAL)

The experiments were carried out on white nonpedigree male mice, each weighing 18-24 g. Compounds VII and X were administered intraperitoneally 30 min before a subcutaneous administration of corazole (100 mg/kg) or application of a maximal electrical shock. The antihypoxic activity on a model of hypoxia with hypercapnia was determined from the increase in the survival time of the animals in a restricted space (210 ml). For comparison, the antihypoxic activity was also determined for compound I (n = 5), the antispasmodic activity of which was studied previously [1]. The acute toxicity was determined at a single intraperitoneal administration of the compounds. The number of animals that died in the course of 10 days was taken into account. The experimental results were processed statistically ($p \leq 0.05$) [1].

Acid VII, which can be regarded as an analog of the "inactive" 2-amino-6-phosphonohexanoic acid I (n = 4) displayed an antihypoxic and a high antispasmodic effect. According to the electrical shock test, the antispasmodic activity of compound VII was rated as ED_{50} 89.6 mg/kg. This value is somewhat higher than the ED_{50} (64.8 mg/kg) for 2-amino-7-phosphonoheptanoic acid I (n = 5) the most active representative of the PAA series, which we had previously studied [1]. The spectrum of the antispasmodic activity of comopund VII is probably the same as that of compound I (n = 5), i.e., the presence of antispasmodic activity accordig to the maximal electrical shock test, and absence of anticorazole activity [1].

Acid VII also has a certain antihypoxic activity. In a dose approximately equal to its ED_{50} for the maximal electrical shock (100 mg/kg), it increases the survival time of mice by 26%. Increase in the survival time is also observed for compound I (n = 5): in a dose of 65 mg/kg (the ED_{50} for the maximal electrical shock) it causes an increase in the survival time by 15%, and in a dose of 130 mg/kg – by 30%.

Compound X is like a twin molecule of compound VII, but in contrast to it, does not have an antispasmodic and antihypoxic activity in doses of up to 300 mg/kg. The toxicity of acids VII and X is low – the LD_{50} is more than 1000 mg/kg.

Thus, the results of the investigations showed that the introduction of the o-xylylene fragment into the molecule of PAA leads to a considerable intensification of the antispasmodic activity, compared with the inactive formal prototype I (n = 4),and even in comparison with acid I (n = 3) (ED_{50} 295 mg/kg according to the maximal electrical shock test). It is possible that the distance between the functional groups in VII is closer to that in acid I (n = 3). In this case, the increase in the lipophilicity is an accompanying positive factor, increasing the permeability of compound VII through the hematoencephalic barrier, and thus also its antispasmodic effect, compared with acid I (n = 3).

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