Synthesis of phosphinic and phosphonic analogs of aspartic acid

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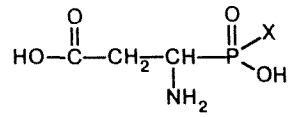
Approaches to the synthesis of 1-amino- and 2-amino-2-carboxyethylphosphinic and -phosphonic acids have been studied. A convenient method for the preparation of phosphinic acids is the reactions of ethyl diethoxymethylphosphonite with ethyl acetamidomethylenemalonate and ethyl 2-acetamidoacrylate.

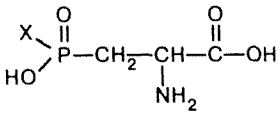
Key words: organophosphorus analogs of amino acids, 1-amino-2-carboxyethylphosphinic acid, 2-amino-2-carboxyethylphosphinic acid.

In the series of biologically active phosphorus-containing analogs of amino acids (**1**—**4**), the family of analogs of aspartic acid is of special interest.



1, 2

1: X = H, **2**: X = OH



3, 4

3: X = H, **4**: X = OH

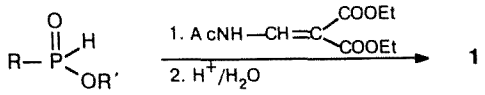
Compounds **3** and **4** have been found in nature,[1] and acid **3** produced by micromycetes exhibits antiviral activity. The analogs of the inhibitor of pyrimidine biosynthesis were obtained[2] on the basis of 3-phosphono-β-alanine (**2**). These compounds and their derivatives bind specifically to neuroreceptors. The possibility of an active participation of aspartic acid analogs **1**—**4** in the nitrogen metabolism is in accordance with their substrate properties in the aspartate-aminotransferase reaction.[3]

In the present work, new methods for the preparation of phosphorus-containing analogs of aspartic acid, first of all, phosphinic acids **1** and **3** and their α-Me-derivatives, the possibility of their derivation, and their transformations into the corresponding phosphonates have been studied.

α-Aminoalkylphosphinic acids are usually obtained by the addition of H_3PO_2,[4] $HP(OSiMe)_2$,[5,6] or $(EtO)_2CHP(O)(H)OEt$ [7] to the C=N bond of *N*-substituted imines followed by the removal of protecting groups and by the reaction of oximes with H_3PO_2.[8] Aminophosphinic acids **1** and **3** have been previously obtained by cumbersome multistage syntheses.[9,10]

We have developed a method for the synthesis of aminophosphinic acids **1** and **3** based on diethoxymethylphosphonite (**5**) obtained from methyl orthoformate and hypophosphorous acid by the known procedure.[11] The reaction of phosphonite **5** with ethyl acetamidomethylenemalonate[12] under standard conditions of the Michael reaction followed by acid hydrolysis gives product **1** in a higher yield than that attained by the known methods. The use of methyl hypophosphite (**6**) as a hydrophosphoryl synthon in this reaction results in the formation of a complex mixture of products, from which amino acid **1** was isolated in 6.5 % yield (Scheme 1).

Scheme 1



5, 6

5: R = $(EtO)_2CH$; R′ = Et

6: R = H; R′ = Me

Amino acid **3** was successfully synthesized by the reaction of ester **5** with ethyl 2-acetamidoacrylate

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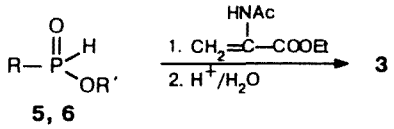
(Scheme 2), and the yield of product **3** is twice as great as the reported yield. As in the case of amino acid **1**, when ester **6** was used, the yield of amino acid **3** decreases sharply and becomes equal only to 8.5 %.

Scheme 2



We have also studied the possibility of using the reactions of oximes with hypophosphorous acid for the preparation of phosphinic analogs of aspartic acid. Under standard conditions of this reaction, formylacetic acid oxime mainly undergoes decarboxylation, which results in the formation of α-aminoethylphosphinic acid (**7**) as the main product, while the yield of acid **1** was 1.6 %. Ethyl acetoacetate oxime reacts similarly to give mainly 1-amino-1-methylethylphosphinic acid (**8**),* while amino acid (**9**) is obtained in a low yield (Scheme 3).

It is noteworthy that compounds **8** and **9** are the first representatives of the previously unknown phosphinic analogs of α-substituted amino acids.

Derivatives of phosphinic analogs of aspartic acid have not been described yet. Among them, the carboxy group derivatives, for example, an analog of asparagine, are of special interest. The formation of the corresponding amide was observed in preliminary experiments on the ammonolysis of ester **1** obtained under standard conditions of esterification of amino carboxylic acids. The synthesis of various derivatives of phosphinic analogs of aspartic acid is a subject of our studies and will be published elsewhere.

The oxidation of acids **1**, **3**, and **8** by bromine in an acidic medium results in the formation of aminophosphonates **2**, **4**, and $Me_2C(NH_2)P(O)(OH)_2$ (**10**), respectively. The presence of the carboxy group at the β-position does not complicate the reaction, and the yields, as for the majority of the described phosphinic analogs of proteinogenic amino acids,[4,8] are close to quantitative ones. Thus, the oxidation can be considered as an alternative method for the preparation of phosphonic analogs of aspartic acid and its derivatives and as an independent confirmation of the structure of the corresponding aminophosphonites **1** and **3**.

The synthesis of aminophosphonic acid **2** was also performed by the Arbuzov reaction from ethyl (chlorocarbonyl)acetate and tribenzyl phosphite. Subsequent hydrogenolysis of the α-oxophosphonate formed, saponification, and reductive amination result in the product **2**. This method can be applied for the preparation of acid **2** containing the tritium label at the α-position.

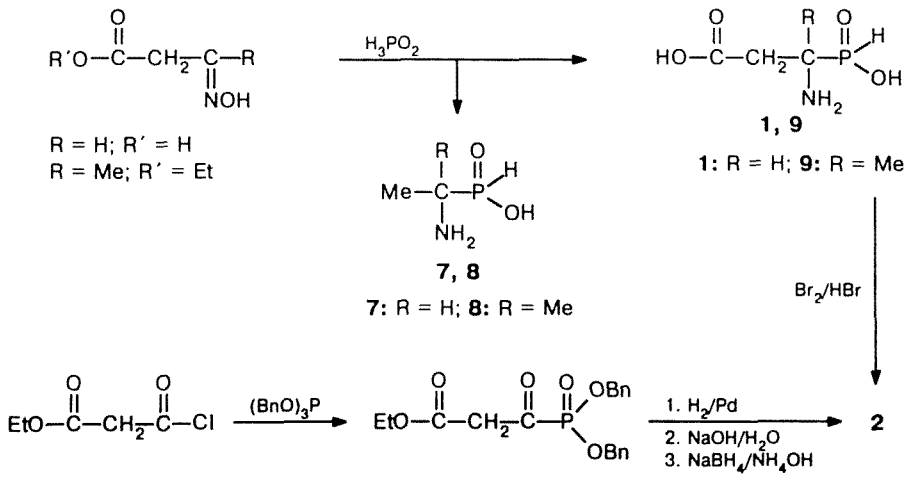
Compound **4** was prepared by the addition of diethyl phosphite to ethyl acetamidoacrylate in the presence of a basic catalyst followed by acid hydrolysis, which made it possible to synthesize compound **4** in a higher yield than that reported.[16]

Experimental

TLC was carried out on Silufol UV_{254} plates in the solvent systems *A* (Pr^iOH—25 % NH_4OH—H_2O, 7 : 1 : 2) and *B* ($MeOH-H_2O-25\ \%\ NH_4OH-CF_3COONH_4$, 120 : 49 : 30 : 1); compounds were detected by color reactions with ninhydrin and ammonium molybdate. Ion-exchange chromatography was carried out on a Dowex-50×8 cation-exchange resin (100—200 mesh, H^+-form) (BioRad), aqueous 15 % Pr^iOH was used as eluent. Melting points were determined on an Electrothermal instrument and were not cor-

* This acid was also obtained by the reaction of hypophosphorous acid with acetone oxime.

Scheme 3



rected. ^{1}H NMR spectra were recorded on a Varian XL-100-15 instrument (relative to Bu^{t}OH). Mass spectra were obtained by the plasma desorption method on an MSBKh instrument.

3-Hydrohydroxyphosphoryl-β-alanine (1). *A.* A solution of 2.0 *M* MeONa (1.3 mL) in MeOH was added to a mixture of ethyl acetamidomethylenemalonate (5.73 g, 25 mmol) and ester **5** (6.1 g, 32 mmol), and the temperature raised from 20 to 55 °C. The mixture was left for 3 days at 20 °C, and 20 % HCl (100 mL) was added. The mixture was refluxed for 3 h in an Ar atmosphere and concentrated to dryness *in vacuo*. The residue was dissolved in 15 % Pr^{i}OH, and product **1** was isolated by ion-exchange chromatography. Acid **1** (1.4 g, 36 %) was obtained, m.p. 219 °C (decomp., H_2O—EtOH) [*cf.* Ref. 10: 230—231 °C (decomp.); Ref. 9: 209—212 °C (decomp.)]. R_f 0.32 (*A*); 0.80 (*B*). ^{1}H NMR (D_2O), δ: 2.53—3.10 (m, 2 H, CH_2); 3.30—3.58 (m, 1 H, CH); 6.73 (dd, 1 H, PH, ^1J = 509 Hz). Mass spectrum, *m/z*: 152.5 $[M-1]^+$.

B. $HC(OMe)_3$ (1.06 g, 10 mmol) was added to H_3PO_2 (0.66 g, 10 mmol) in an Ar atmosphere at 0 °C with stirring. After 3 h, a solution of ethyl acetamidomethylenemalonate (2.30 g, 10 mmol) in dioxane (5 mL) was added followed by solid MeONa to pH 8. The mixture was stirred for 16 h at 20 °C, MeONa being added intermittently, and then 10 % HCl (20 mL) was added. The mixture was refluxed for 3 h in an Ar atmosphere and concentrated to dryness *in vacuo*. The residue was dissolved in 15 % Pr^{i}OH, and product **1** was isolated as described in method *A*. Compound **1** (0.1 g, 6.5 %) was obtained after recrystallization from aqueous EtOH.

C. Formylacetic acid oxime[13] (0.82 g, 8 mmol) was added slowly to H_3PO_2 (1.05 g, 16 mmol) in an Ar atmosphere with stirring, and the temperature was maintained at 20—24 °C. After 16 h at 20 °C, the mixture was diluted with 15 % Pr^{i}OH, and the products were isolated by ion-exchange chromatography. Amino acid **1** (20 mg, 1.6 %) and amino acid **7** (117 mg, 12 %) identical with the authentic sample[8] were obtained.

3-Hydrohydroxyphosphoryl-α-alanine (3). *A.* A solution of 2.0 *M* MeONa (0.5 mL) in MeOH was added with stirring to a mixture of ethyl acetamidoacrylate (3.2 g, 20 mmol) and ester **5** (4.88 g, 25 mmol), while the temperature increased from 20 to 65 °C. The mixture was kept for 3 days at 20 °C, and 20 % HCl (75 mL) was added. The mixture was refluxed for 3 h in an Ar atmosphere and concentrated *in vacuo* to dryness. The residue was dissolved in 15 % Pr^{i}OH, and acid **3** was isolated by ion-exchange chromatography, yield 1.82 g (60 %), m.p. 208—210 °C (decomp., from H_2O—EtOH) [*cf.* Ref. 10: m.p. 200 °C (decomp.) for semihydrate], R_f 0.25 (*A*); 0.77 (*B*). Found (%): C, 23.68; H, 5.28; N, 9.09; P, 20.41. $C_3H_8NO_4P$. Calculated (%): C, 23.54; H, 5.27; N, 9.15; P, 20.23. ^{1}H NMR (D_2O), δ: 1.80—2.45 (m, 2 H, CH_2); 3.92— 4.40 (m, 1 H, CH); 6.83 (dd, 1 H, PH, ^1J = 506 Hz). Mass spectrum, *m/z*: 152.2 $[M-1]^+$.

B. $HC(OMe)_3$ (2.12 g, 20 mmol) was added to H_3PO_2 (1.32 g, 20 mmol) in an Ar atmosphere at 0 °C with stirring. After 3 h, a solution of ethyl acetamidoacrylate (3.2 g, 20 mmol) in dioxane (5 mL) was added, and then solid MeONa was added to pH 8 (the mixture was warmed to 30 °C). The mixture was stirred for 16 h at 20 °C with intermittent addition of MeONa, then 10 % HCl (20 mL) was added, the mixture was refluxed for 3 h in an Ar atmosphere and concentrated to dryness *in vacuo*. The residue was dissolved in 15 % Pr^{i}OH, and product **3** was isolated as described in method *A*. Amino acid **3** (0.26 g, 8.5 %) was obtained after recrystallization from aqueous EtOH.

1-Amino-1-methylethylphosphinic acid (8). A solution of acetone oxime (7.3 g, 0.1 mol) in Pr^{i}OH (15 mL) was added over 30 min to a boiling solution of H_3PO_2 (13.2 g, 0.2 mol) in Pr^{i}OH (85 mL), and the mixture was refluxed for 2 h. Then, one more portion of acetone oxime (7.3 g, 0.1 mL) in Pr^{i}OH (15 mL) was added over 30 min (refluxing was continued), and the mixture was refluxed for 4 h. The mixture was cooled, and the crystals precipitated were filtered off and washed with Pr^{i}OH. The combined filtrates were neutralized with Et_3N to pH ≈ 4.0, and an additional amount of the product was filtered off on cooling. The product was recrystallized from H_2O to obtain acid **8** (9.0 g, 36.5 %), m.p. 222—224 °C (decomp., from H_2O). R_f 0.42 (*A*); 0.79 (*B*). ^{1}H NMR (D_2O), δ: 1.42 (d, 6 H, Me, ^3J = 13 Hz); 6.85 (d, 1 H, PH, ^1J = 519 Hz). Mass spectrum, *m/z*: 122.1 $[M-1]^+$.

3-Amino-3-hydrohydroxyphosphorylbutyric acid (9). A solution of ethyl acetoacetate oxime [from ethyl acetoacetate (37.0 g, 0.25 mol) and NH_2OH (8.25 g, 0.25 mol)] in EtOH was added slowly to a boiling solution of H_3PO_2 (33 g, 0.5 mol) in anhydrous EtOH (100 mL), and the mixture was refluxed for 21 h. The mixture was concentrated to dryness, and the residue was dissolved in 15 % Pr^{i}OH. A mixture of aminophosphinic acid **8** and ethyl ester of amino acid **9** was isolated by ion-exchange chromatography, dissolved in 20 % HCl (160 mL), refluxed for 3 h, and concentrated to dryness. The residue was dissolved in 20 % HCl (160 mL), and the solution was refluxed for 3 h and concentrated to dryness. The residue was dissolved in 15 % Pr^{i}OH, and products were isolated by ion-exchange chromatography. Aminophosphinic acid **8** (1.73 g, 5.6 %) identical to the authentic sample and product **9** [(0.29 g, 0.7 %, m.p. 210—211 °C (decomp., from H_2O—EtOH)] were isolated. R_f 0.21 (*A*); 0.72 (*B*). ^{1}H NMR (D_2O), δ: 1.49 (d, 3 H, Me, ^3J = 14 Hz); 2.65—2.80 (m, 2 H, CH_2); 6.60 (d, 1 H, PH, ^1J = 506 Hz). Mass spectrum, *m/z*: 167.3 $[M-1]^+$.

3-Phosphono-α-alanine (4). A solution (0.1 mL) of sodium diethyl phosphite [from diethyl phosphite (0.5 mL) and Na (0.05 g)] was added to a mixture of ethyl acetamidoacrylate[15] (2.8 g, 18 mmol) and diethyl phosphite (2.8 g, 20 mmol), and the mixture was left overnight at 20 °C. Then the mixture was neutralized with AcOH, and the volatiles were distilled off *in vacuo* (1 Torr). 20 % HCl (35 mL) was added to the residue, and the mixture was refluxed for 4.5 h. The mixture was concentrated to dryness, and then H_2O (30 mL) was added and evaporated twice. The residue was dissolved in EtOH, and propylene oxide was added. The crude product (2.05 g) was obtained. The final purification was carried out by ion-exchange chromatography to obtain product **4** (1.6 g, 53 %): m.p. 225 °C (decomp., from H_2O) [*cf.* Ref. 16: m.p. 228 °C (decomp.)]. Found (%): C, 21.13; H, 4.70; P, 18.26. $C_3H_8NO_5P$. Calculated (%): C, 21.32; H, 4.77; P, 18.33; R_f 0.06 (*A*); 0.40 (*B*). ^{1}H NMR (D_2O), δ: 1.87—2.50 (m, 2 H, CH_2); 3.88—4.20 (m, H, CH).

3-Phosphono-β-alanine (2). *A.* A solution of dibenzyl 2-(ethoxycarbonyl)-1-oxoethylphosphonate[14] (3.8 g, 10 mmol) in EtOH (30 mL) was hydrogenolyzed over Pd black at atmospheric pressure. The catalyst was filtered off and washed with EtOH, and the combined filtrates were concentrated to half the initial volume. A 5 *M* aqueous solution of NaOH (6.0 mL) was added dropwise with stirring to the solution obtained. The solution was stirred for 30 min at 20 °C and concentrated to dryness *in vacuo*. The residue was dissolved in an aqueous solution of NH_3 (60 mL) saturated at 0 °C and was stirred for 1 h at 0 °C. $NaBH_4$ (0.378 g, 10 mmol) was added in small portions for 1 h, and the solution was stirred for 2 h at 0 °C and concentrated to dryness *in vacuo*. The residue was dissolved in 1.0 *M* HCl (50 mL), the solution was concentrated to dryness *in vacuo*, and the residue was dissolved in 15 %

Pr^{i}OH. Product **2** (0.42 g, 25 %) was isolated by ion-exchange chromatography: m.p. 221 °C (decomp., from H_2O—EtOH) [*cf.* Ref. 12, m.p. 234—238 °C (decomp.)]. Found (%): C, 21.37; H, 5.03; P, 18.29. $C_3H_8NO_5P$. Calculated (%): C, 21.32; H, 4.77; P, 18.33. R_f 0.05 (*A*); 0.38 (*B*). ^{1}H NMR (D_2O), δ: 2.50—3.15 (m, 2 H, CH_2); 3.40—3.70 (m, H, CH).

B. Br_2 (0.6 mL, 11.8 mmol) was added dropwise with stirring to a solution of amino acid **1** (1.53 g, 10 mmol) in 1 *M* HCl (10 mL), and the mixture was stirred for 30 min at 20 °C. The mixture was concentrated to dryness, the residue was dissolved in MeOH, and propylene oxide was added. The crude product (1.65 g) was obtained, which was recrystallized from H_2O to obtain product **2** (1.56 g, 92 %): m.p. 226 °C (decomp., from H_2O—EtOH) [*cf.* Ref. 12: m.p. 234—238 °C (decomp.)]. Found (%): C, 21.37; H, 5.03; P, 18.29; $C_3H_8NO_5P$. Calculated (%): C, 21.32; H, 4.77; P, 18.33. R_f 0.05 (*A*); 0.38 (*B*). ^{1}H NMR (D_2O), δ: 2.50—3.15 (m, 2 H, CH_2); 3.40—3.70 (m, H, CH).

Aminophosphonates **4** and **10** were prepared similarly in 87 and 79 % yields, respectively, from the corresponding aminophosphinic acids **3** and **8**.

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