ORGANOPHOSPHORUS ANALOGS OF BIOLOGICALLY ACTIVE COMPOUNDS

5.* SYNTHESIS OF α-AMINOPHOSPHONIC ACIDS AND SOME OF THEIR DERIVATIVES

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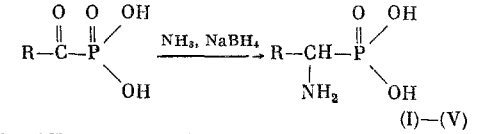
UDC 542.91:547.1'118:541.69

The first aminophosphonic acids were obtained many years ago, but these compounds have only recently found use as amino-acid analogs in enzymological research. Liver preparations are capable of converting α-ketoglutarate into glutamate in the presence of some α-aminophosphonic acids [2]. The phosphonate analog of phenylalanine was found to be a specific inhibitor of phenylalanyl-tRNA-synthetase [3], while α-aminoethylphosphonic acid has the same strong effect on the activity of D-alanine racemase as the cycloserine antibiotics [4]. Thus, the need arose for new methods for the synthesis of aminophosphonic acids, the synthesis of new representatives of this compound class, and a study of the transformations of these compounds.

The methods known for the preparation of aminophosphonic acids are largely analogous to the syntheses of aminocarboxylic acids [5]. One of the most well-studied methods involves the use of esters of α-ketophosphonic acids as starting materials which are converted by a series of steps into α-aminophosphonates [6]. We carried out a one-step synthesis of α-aminophosphonic acids according to the method developed for the preparation of α-ketophosphonic acids [1]:

Scheme 1

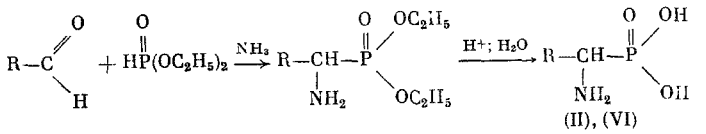


R=CH3 (I); (CH3)2CH (II), (CH3)2CHCH2 (III); C6H5CH2 (IV); HOCOCH2CH2 (V)

In aqueous or alcoholic solutions of ammonia, α-ketophosphonic acids but not their di- or monoesters are converted by the action of sodium borohydride into α-aminophosphonic acids with yields from 50 to 70%. This method was used both for the synthesis of (I)-(IV) which were previously known and the new phosphonate analog of glutaminic acid (V). An advantage of this method is the possibility of effecting the facile preparation of tritium-labeled aminophosphonates required for enzymological research, for example, α-^{3}H-(II) [7]. Primary amines may be used in the reductive amination instead of ammonia, as shown in the preparation of N_α-methyl-α-amino-β-phenylethylphosphonic acid (N_α-CH_3-(IV)).

A simple method for the synthesis of α-aminophosphonic acids is the aminophosphonylation of aldehydes which is carried out by the heating of aldehydes (such as propionaldehyde and benzaldehyde) with diethyl phosphite in ethanolic solutions of ammonia with subsequent hydrolysis of the aminophosphonate diesters [8]. In this manner, we obtained only traces of (II), though the yield of (II) was 16% in the reaction in liquid ammonia at 80°C.

Scheme 2



(II), (VI)

R = (CH3)2CH (II); CH3SCH2CH2 (VI)

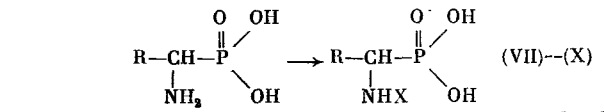
*Previous communication [1].

Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 9, pp. 2118-2122, September, 1979. Original article submitted May 5, 1978.

The synthesis of (VI), a previously unknown analog of methionine, was carried out similarly.*

Relative to the chemistry of amino acid derivatives, the chemistry of aminophosphonic acid derivatives has hardly been developed. For subsequent syntheses in this series, we obtained N-formyl, N-trifluoroacetyl, and N-carbobenzoxy derivatives of aminophosphonic acids.

Scheme 3

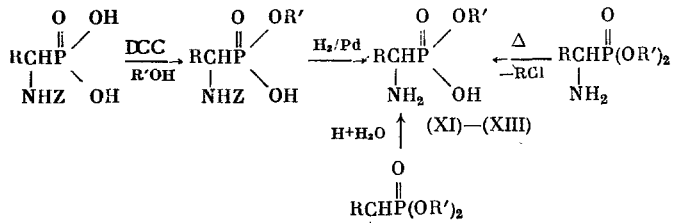


R = $(CH_3)_2CH$; X = $C_6H_5CH_2OCO$ (VII); R = $(CH_3)_2CH$; X = CF_3CO (VIII).
R = $C_6H_5CH_2$; X = $C_6H_5CH_2OCO$ (IX); R = $C_6H_5CH_2$; X = HCO (X).

The conditions were analogous to those used for amino acids, but longer periods were required to complete these reactions.

The synthesis of aminophosphonate monoesters was carried out by several methods for (II) and (IV):

Scheme 4

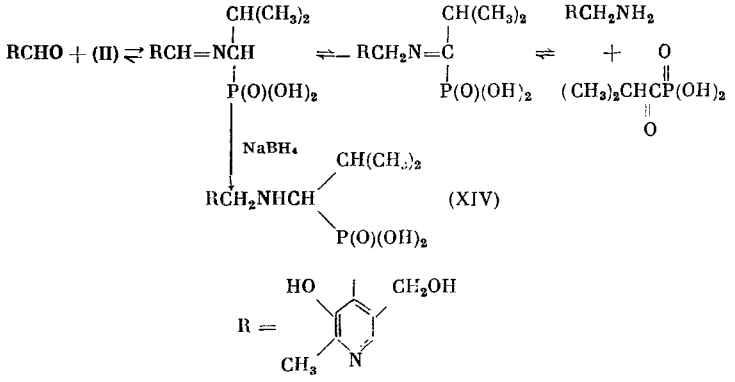


R = $(CH_3)_2CH$, R′ = CH_3 (XI); R = $(CH_3)_2CH$, R′ = C_2H_5 (XII); R = $C_6H_5CH_2$, R′ = C_2H_5 (XIII).

In one of these methods, heating of the hydrochloride salt of diester (II) led directly to the monoester (XII) in high yield, while in another method, N,N′-dicyclohexylcarbodiimide (DCC) was used for the esterification of (VII) with subsequent removal of the protective group by the usual means, and, finally, monoester (XIII) was obtained by the partial hydrolysis of the diester (IV) in 80% acetic acid.

The basis for the various transformations of amino acids which proceed with the participation of pyridoxal enzymes is their capacity to form aldimines with the pyridoxal-5′-phosphate coenzyme. It was interesting to compare aminocarboxylic and aminophosphonic acids in this regard since the possibility of enzymatic transamination had only been shown for one aminophosphonic acid, namely, β-aminoethylphosphonic acid [9]. A convenient model for nonenzymatic transamination is the reaction of an α-amino acid with pyridoxal in ethanolic solutions [10]. Under these conditions, (II) and pyridoxal rapidly react to form a pyridoxylidene derivative whose UV spectrum is virtually identical to the corresponding derivative of valine.

Scheme 5



*This synthesis was carried out with the participation of A. T. Prudchenko.

The reduction of the reaction mixture by $NaBH_4$ led to a single product, namely, N_α-pyridoxyl-α-aminoisobutylphosphonic acid. The structure of this compound was studied by UV, TLC, and electrophoresis mobilities, positive tests for phosphorus, and tests with ninhydrin and 2,4-dichloroquinonechlorimine. The reaction of valine with pyridoxal after 24 h led to the pyridoxamine in 6.5% yield, while in the case of (II), the yield of pyridoxamine, if formed, was <0.01%. Thus, in the case of aminophosphonic acids, the equilibrium is virtually entirely shifted towards pyridoxal and the amino acid, which is in accord with our results on the complete conversion of pyridoxamine into pyridoxal in the reaction with α-ketophosphonic acids [1].

EXPERIMENTAL

The chromatography was performed on Silufol UV_{254} plates and on FN-18 paper in a system consisting of 7 : 1 : 2 isopropyl alcohol–25% NH_4OH–water. Electrophoresis was performed on FN-18 paper in 0.05 M acetate buffer at pH 4.1 or in 4 : 40 : 756 pyridine–CH_3CO_2H–H_2O buffer at pH 3.5 with voltage gradient 68 V/cm. The substances were visualized by UV absorption and color reactions with ninhydrin, ammonium molybdate, and 2,4-dichloroquinochloroimine (for derivatives of 3-hydroxypyridine). The spectra were taken on a Specord UV-VIS spectrometer. The dibenzyl esters of the α-ketophosphonic acids were obtained according to our earlier work [1].

Reductive Amination of α-Ketophosphonates. The dibenzyl ester of α-ketophosphonic acid (1 mmole) was hydrogenated over palladium black in 5 ml methanol. The filtrate was neutralized with 25% NH_4OH and evaporated in vacuum. Then, 4 ml aqueous NH_4OH (saturated at 0°C) was added to the residue and 1 mmole $NaBH_4$ in 1 ml 25% NH_4OH was added dropwise with cooling and stirring over 15 min. After 1 h at 20°C, the mixture was evaporated in vacuum to dryness, dissolved in 0.5 ml water, acidified with 20% HCl to pH 1, and 5 ml ethanol was added. The filtrates were evaporated in vacuum, dissolved in 5 ml ethanol, and brought to pH 4.5-5 with ethanolic Et_3N. The residue was washed with ethanol, ether, and dried in vacuum. The yields of (II), (III), and (IV) ranged from 50 to 70%. The isolation of (I) was carried out on Dowex 50 × 8 sulfo cation-exchange resin, 100-200 mesh, H^+ form with 0.5 N NH_4OH eluent to provide a 50% yield.

α-Amino-γ-carboxypropylphosphonic Acid (V). To an ethanolic solution of α-keto-γ-carbomethoxypropylphosphonic acid obtained by the hydrogenation of 1 mmole of the dibenzyl ester, 3 mmoles ethanolic NaOH was slowly added, stirred for 30 min at 20°C, and evaporated in vacuum to dryness. The residue was dissolved in 12 ml aqueous NH_4OH (saturated at 0°C) and then the reaction was formed as described above. Acid (V) was isolated by chromatography on Dowex 50 × 8 resin, H^+ form, water elution. The yield of (V) was 90 mg (45%), mp 185-187°C (from water–isopropyl alcohol), Rf 0.01. Found: C 26.25; H 5.44; P 16.94; N 7.68%. Calculated for $C_4H_{10}NO_5P$: C 26.22; H 5.46; P 16.93; N 7.65%.

N_α-Methyl-α-amino-β-phenylethylphosphonic Acid (N-CH_3-(IV)). The reductive aminomethylation of 1 mmole α-keto-β-phenylethylphosphonic acid in 5 ml 33% aqueous methylamine was carried out by the general method. The yield of (IV) was 50%, mp 239-242°C (from water–isopropyl alcohol), R_f 0.24. Found: C 50.86; H 6.35; P 14.75; N 6.97%. Calculated for $C_9H_{14}NO_3P$: C 50.23; H 6.56; P 14.39; N 6.50%.

α-Aminoisobutylphosphonic Acid (II) from Isobutyraldehyde. To 150 ml liquid ammonia, 36 g freshly distilled isobutyraldehyde was added with cooling and stirring and then 69 g diethyl phosphite was added and the mixture was heated in an autoclave for 7 h at 80°C. After removal of the ammonia, the mixture was stirred with 100 ml ether and the residue was washed with 50 ml ether, heated at reflux for 8 h with 200 ml 20% HCl and the mixture was evaporated to dryness in vacuum. The residue was dissolved in 75 ml ethanol and the filtrate was brought to pH 4.5-5 with ethanolic triethylamine and, after 2 h at 0°C, 4 g (II) was filtered off. After removal of the solvent from the ethereal extractions and distillation of the residue in vacuum, 13 g diethyl ester of (II) was obtained, bp 86-88°C (0.5 mm Hg), n_D^{20} 1.4400, hydrochloride salt, mp 116°C (from ethanol–ether).

The hydrolysis of this ester to (II) was performed by heating with an excess of 20% HCl for 6-8 h and subsequent evaporation and neutralization. The total yield of (II) was 16%.

α-Amino-γ-methylmercaptopropylphosphonic Acid (VI). A yield of 10 g crude diethyl ester of (VI) was obtained from 10 g γ-methylmercaptopropionaldehyde [9] and 14.5 g diethyl phosphite in 100 ml liquid ammonia as described above. A yield of 80 mg (10.5%) acid with mp 271-272°C (from water–ethanol) and R_f 0.11 was obtained after 6 h hydrolysis of 1 g diester by heating at reflux with 10 ml 20% HCl and purification on Dowex 50 × 8 resin, H^+ form, 0.5 N NH_4OH elution. Found: C 25.93; H 6.51; P 16.80; N 17.37%. Calculated for $C_4H_{12}NO_3PS$: C 25.96; H 6.54; P 16.74; N 17.30%.

Carbobenzoxylation of α-Aminophosphonic Acids. A mixture of 5 mmoles aminophosphonic acid, 17.5 mmoles $NaHCO_3$, 10 ml water, and 5.5 mmoles carbobenzoxychloride was stirred for 12 h at 20°C until a negative ninhydrin test was obtained and extracted with ether. The aqueous portion was acidified with 20% HCl to pH 1 and the oil which separated out was extracted with dichloroethane (four 5-ml portions). The extracts were washed with water and the solvent was distilled off in vacuum. After drying of the residue in vacuum over P_2O_5, the carbobenzoxy derivatives were obtained. A yield of 1 g (70%) (VII) was obtained as a hygroscopic amorphous substance with R_f 0.40. Found: N 4.77; P 10.63%. Calculated for $C_{12}H_{18}NO_5P$: N 4.87; P 10.80%. A yield of 0.740 g (50%) (IX) was obtained with R_f 0.33. Found: N 4.27; P 9.08%. Calculated for $C_{16}H_{18}NO_5P$: N 4.18; P 9.25%.

N_α-Trifluoroacetyl-α-aminoisobutylphosphonic Acid (VIII). A sample of 5 mmoles (II) was added to 2 ml trifluoroacetic anhydride and stirred until complete dissolution and then evaporated to dryness in vacuum. The residue after drying in vacuum over KOH was dissolved in hot ethyl acetate and the filtrate was evaporated in vacuum to 5-7 ml. Then, 10 ml warm heptane was added and the solution was left to crystallize at 4°C. A yield of 0.84 g (VIII) (65%) was obtained with mp 235-238°C and R_f 0.45. Found: C 29.05; H 4.20; P 12.50; N 5.53%. Calculated for $C_6H_{11}NO_4F_3P$: C 28.90; H 4.41; P 12.45; N 5.62%.

N_α-Formyl-α-aminophenylethylphosphonic Acid (X). To 1 mmole (IV) in 2.5 ml 100% formic acid at 4°C, 0.7 ml acetic anhydride was added and let stand at 20°C for 48 h. Then 1 ml cold water was added and the solution was evaporated in vacuum at 40°C to dryness. The yield of (X) was 0.21 g (80%), mp 196-204°C (dec.) (from ethanol–ether), R_f 0.17. Found: C 47.60; H 5.18; P 13.54; N 5.80%. Calculated for $C_9H_{12}NO_4P$: C 47.20; H 5.20; P 13.55; N 6.15%.

Monomethyl Ester of α-Aminoisobutylphosphonic Acid (XI). A solution of 1 mmole (VII), 1.5 mmole N,N-dicyclohexylcarbodiimide, and 1 ml methanol in 2.5 ml THF was heated at reflux for 6.5 h taking precautions to exclude atmospheric moisture. After filtration, the solution was evaporated in vacuum to dryness and the residue was dissolved in ether. Then, the filtrate was evaporated in vacuum and the residue obtained was dissolved in 2 ml methanol, acidified with acetic acid, and hydrogenated over 10 mg palladium black. The catalyst was filtered off and washed with 2 ml methanol. The filtrate was evaporated in vacuum at 40°C and the residue was dried in vacuum over P_2O_5. The yield of (XI) was 0.1 g (50%), mp 218-220°C, R_f 0.55. Found: C 36.03; H 8.20; P 18.68; N 8.22%. Calculated for $C_5H_{14}NO_3P$: C 35.92; H 8.38; P 18.56; N 8.38%.

Monoethyl Ester of α-Amino-β-phenylethylphosphonic Acid (XIII). A solution of 10 mmoles diethyl ester of (IV) in 100 ml 80% acetic acid was heated at reflux for 20 h and evaporated in vacuum. The residue was dried in vacuum over KOH and P_2O_5. The yield of (XIII) was 0.2 g (10%), mp 232-234°C (from water–dioxane), R_f 0.64. Found: 50.53; H 6.81; P 13.60; N 5.61%. Calculated for $C_{10}H_{16}NO_3P$: C 50.46; H 7.10; P 13.02; N 5.88%.

Monoethyl Ester of α-Aminosiobutylphosphonic Acid (XII). A sample of 1 mmole hydrochloride salt of the diethyl ester of (II) was heated for 20-30 min at 120-125°C until the melt solidified and then held at this temperature in vacuum for an additional 30 min (negative Cl test). The yield of (XII) was 0.15 g (85%), mp 225-228°C (from ethanol–2-propanol), R_f 0.53. Found: C 39.68; H 8.71; P 11.93; N 7.60%. Calculated for $C_6H_{16}NO_3P$: C 39.55; H 8.83; P 11.60; N 7.73%.

Nonenzymatic Transamination of (II). To 0.05 mmoles (II) in 10 ml 0.005 M NaOH in abs. methanol, 0.05 mmole pyridoxal hydrochloride was added and the mixture was stirred for 24 h with precautions to exclude atmospheric moisture and light. Aliquots were taken after 30 min, 2 h, and 6 h and were diluted with 0.1 M ethanolic NaOH and the UV spectra were taken. After 24 h, the amount of pyridoxamine formed was determined. For this determination, 5 ml of the reaction mixture was evaporated to dryness in vacuum, the mixture was separated by electrophoresis at pH 3.5, and the electrophoresis patterns were scanned at 330 nm. A parallel run with L-valine was performed.

N_α-Pyridoxyl-α-aminoisobutylphosphonic Acid (XIV). To an ethanolic solution of the pyridoxylidene derivative was prepared as described above, 0.05 mmole $NaBH_4$ was added in portions with stirring and after 2 h, was acidified with ethanolic HCl and filtered. After evaporation in vacuum, a yield of 0.3 g (XIV) was obtained with λ_{max} 327 nm (pH 7.0) which is uniform upon electrophoresis (pH 4.1, $E^*_{PM} = -0.2$, detection by ninhydrin, ammonium molybdate, and 2,4-dichloroquinochloroimine and fluoroescence in UV light.

CONCLUSIONS

1. A new method has been proposed for the synthesis of α-amino- and α-alkylaminophosphonic acids.

*The value of the electrophoretic mobility of the product relative to the electrophoretic mobility of pyridoxamine (PM).

2. N-formyl, N-trifluoroacetyl, and N-carbobenzoxy derivatives of α-aminophosphonic acids were synthesized.

3. Methods were developed for obtaining monoesters of α-aminophosphonic acids.

4. Formation of pyridoxamine from pyridoxal and α-aminoisobutylphosphonic acid was not found under conditions for nonenzymatic transamination.

LITERATURE CITED

1. R M. Khomutov, T. I. Osipova, and Yu. N. Zhukov, Izv. Akad. Nauk SSSR, Ser. Khim., 1391 (1978).
2. E. Roberts, D. G. Simonsen, M. Horiguchi, and J. S. Kittredge, Science, 159, 886 (1968).
3. J. W. Anderson and L. Fowden, Chem. Biol. Interact., 2, 53 (1970).
4. E. Adams, K. L. Mukherjee, and H. C. Dunathan, Arch. Biochem. Biophys., 165, 126 (1974).
5. K. A. Petrov, V. A. Chauzov, and T. S. Erokhin, Usp. Khim., 43, 2045 (1974).
6. K. D. Berlin, N. K. Roy, and R. T. Claunch, J. Am. Chem. Soc., 90, 4494 (1968).
7. R. M. Khomutov and T. I. Osipova, Izv. Akad. Nauk SSSR, Ser. Khim., 1110 (1979).
8. M. E. Chalmers and G. M. Kosolapoff, J. Am. Chem. Soc., 75, 5278 (1953).
9. J. M. La Nause and H. Rosenberg, Biophys. Biochim. Acta, 165, 438 (1968).
10. J. Matsuo, J. Am. Chem. Soc., 79, 2016 (1957).

BROMINATION OF π-CYCLOPENTADIENYL-π-(3)-1,2-DICARBOLLYLIRON(III) AND ITS BROMO DERIVATIVES

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UDC 542.944:546.14:541.49:547.1'13:547.1'127

The bromination of metallocarboranes has been studied earlier for the example of the cobaltocarboranes [1-3]. With the aim of studying the direction of the reaction and the electron-density distribution in the molecule of π-cyclopentadienyl-π-(3)-1,2-dicarbollyliron(III), compound (I), we have studied its electrophilic bromination. In CH_2Cl_2, (I) is brominated by 1 mole of Br_2 under mild conditions (at 20°C and without a catalyst). In this case, the bromo derivative (II) of green color, in which the Br atom is bonded with the B atom of the dicarbollyl ligand in position 8 (Fig. 1), is formed with good yield:

$$\underset{\text{(I)}}{C_5H_5FeC_2H_2B_9H_9} + Br_2 \xrightarrow[CH_2Cl_2]{20^\circ} \underset{\text{(II)}}{C_5H_5FeC_2H_2B_9H_8\text{—}8\text{-}Br}$$

Evidently, bromination proceeds by an ionic mechanism under these conditions, since the reaction goes in an analogous manner in a medium of AcOH and CH_3NO, where a radical mechanism is but slightly probable. During the bromination of (I) in CH_2Cl_2 in the presence of $AlCl_3$ at −15°, likewise, only (II) is formed. At 20° bromination proceeds less selectively, and along with (II), which is formed in predominating amount, no dibromo or tribromo derivatives are obtained. Further bromination of (II) in the absence of a catalyst does not proceed. Upon bromination of the monobromide (II) by 1 mole of Br_2 in the presence of $AlCl_3$ in CH_2Cl_2 at 20°, the dibromide (III) of green color, in which the Br atoms are bonded with B atoms in positions 8 and 9, is formed as the main product. In addition, an insignificant amount of the tribromide (IV) of green color with Br atoms in the 8, 9, and 12 positions, is present in the reaction mixture:

$$\text{(II)} + Br_2 \xrightarrow[CH_2Cl_2,\ AlCl_3]{20^\circ} \underset{\text{(III)}}{C_5H_5FeC_2H_2B_9H_7\text{—}8,9\text{-}Br_2} + \underset{\text{(IV)}}{C_5H_5FeC_2H_2B_9H_6\text{—}8,9,12\text{-}Br_3}$$

The action of Br_2 on (IV) in CH_2Cl_2 in the presence of $AlCl_3$ during prolonged heating does not allow the introduction of one more Br atom. It should be noted that the electrophilic bromination of o-carborane results in 8,9,10,12-tetrabromo-o-carborane [4]. Substitution in the cyclopentadienyl ligand does not occur during the

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Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 9, pp. 2122-2128, September, 1979. Original article submitted June 16, 1978.

0568-5230/79/2809-1953$07.50