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α-Hydroxy-α-aminophosphinic Acids: I. Synthesis of a New Analog of Phenylglycine and Its Enantiomers

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Abstract — Methods for synthesis of a new phosphinic analog of phenylglycine, (α-aminobenzyl)(hydroxymethyl)phosphinic acid and its enantiomers are developed.

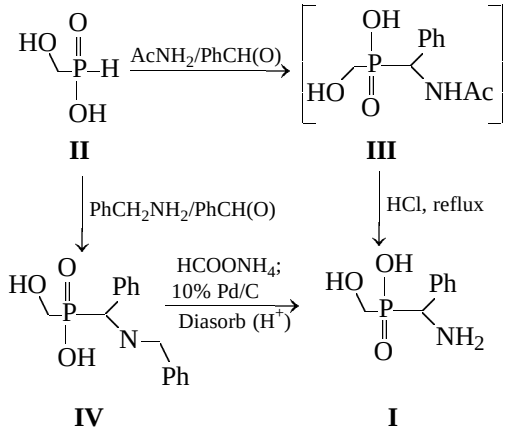
Phosphinic acids containing α-hydroxy and α-amino groups are phosphorus analogs of α-amino as well as α-hydroxy acids. Replacement of the carboxy group in an amino acid by phosphoryl usually results in inhibition of enzymatic processes involving the prototype natural amino acid [1, 2]. Appearance in an α-aminophosphoryl compound of an α-hydroxy group may affect the inhibiting properties of this compound. α-Hydroxy-α-aminophosphinic acids can be regarded, from the one side, as Kabachnik–Fields reaction products and, from the other, as products of the Abramov reaction that is essentially reversible. The ability of α-hydroxyphosphoryl compounds to reversible dissociation [3, 4] with formation of an aminophosphonous component susceptible to oxidation may significantly affect the ability of α-hydroxy-α-aminophosphinic acids to inhibit natural enzymes and alter not only the rate, but also direction of the corresponding biochemical reactions.

Among α-aminophosphoryl compounds, analogs of phenylglycine, especially phosphonic [5, 6] and phosphonous analogs [7, 8], are the most thoroughly studied. These compounds exhibit diverse and significant biologic activity [9, 10]. In this connection of interest is the acidic phosphinic analog of phenylglycine, containing an α-hydroxymethyl fragment.

Here we present various methods for preparing a new phosphinic analog of phenylglycine, (α-aminobenzyl)(hydroxymethyl)phosphinic acid (**I**), and its enantiomers.

We offer a one-pot synthesis of acid **I** from (hydroxymethyl)phosphonous acid (**II**) and acetamide in acetic anhydride by a modified Kabachnik–Fields reaction [11] without isolation of [α-(acetylamino)benzyl]phosphinic acid (**III**) [Scheme (1)]. Besides, as an alternative approach, we propose the synthesis of amino acid **I** by the Kabachnik–Fields reaction with benzylamine as the amino component, followed by hydrogenation of [α-(benzylamino)benzyl]phosphinic acid (**IV**) using ammonium formate as the source of hydrogen [12, 13] [Scheme (1)].

Scheme 1.



The enantiomers of **I** were prepared with the aid of Penicillinamidase (PcAm) [14, 15] as the biocatalyst for hydrolysis of *N*-phenylacetyl derivative **V** which, in its turn, was obtained by the acylation of amino acid **I** with phenylacetyl chloride according to a classical Schotten–Baumann procedure [16] [Scheme (2)].

The (*S*)-**I** enantiomer obtained by enzymatic hydrolysis was separated from unhydrolyzed acid (*R*)-**V** by means ion-exchange chromatography [14, 15]. Acid hydrolysis usually does not touch bonds surrounding chirality center in various phenylglycine analogs [17–19]. Therefore, (*R*)-**V** hydrolyzed without appreciable racemization and gave the optical antipode (*R*)-**I**.

The absolute configuration of the enantiomers of the phosphonic analog of phenylglycine is known [20], and it is unlikely that the presence of a hydroxymethyl

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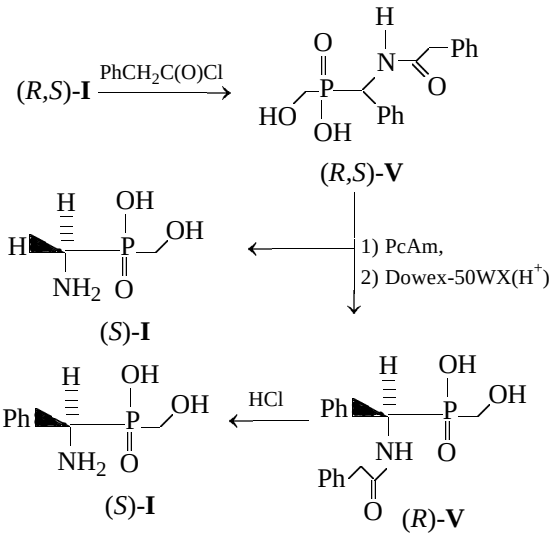
Scheme 2.



fragment on phosphorus may radically alter the configuration of the stereogenic α-carbon atom.

To conclude, methods for synthesis of a new phosphinic analog of phenylglycine (racemate) and an enzymatic method of separation of the amino acid into optical antipodes are offered.

EXPERIMENTAL

The ^1H and ^{31}P NMR spectra were recorded on a Bruker DPX-200 Fourier spectrometer against internal TMS and external 85% H_3PO_4. The optical rotation was measured on Perkin–Elmer 421 and Polamat A polarimeters. The melting points were measured on a Boetius-PHMK device or in a heating block in an open capillary. Thin-layer chromatography was carried out on Silufol plates (eluent chloroform–acetone, 5:1) and on glass plates (Merck) with a UV-254 silica gel layer (0.2 mm); development in UV light or iodine vapor. In addition, Alufol (Kavalier) plates (neutral alumina on aluminum, eluent isobutanol–acetic acid–water, 5:1:1, or pyridine–acetic acid–water–isobutanol, 1:3:5:5) was used. The plates were sprayed with ninhydrin, and amino acids were detected at 100°C. The starting hydroxymethylphosphonous acid was prepared according to the procedure described in [21]. Ion-exchange chromatography was carried out on Dowex 50WX8-200 (H^+) (Lancaster), KU-2-8-U (H^+) (IKhT), and Diasorb-Sulfo (H^+) (BioKhimMak) cation-exchange resins.

Enzymatic hydrolysis was followed and quantitative analysis of the mixture was carried out by means of HPLC on a Gilson chromatograph with a UV-Vis 118 detector at 254 nm, column Diasorb 130 C16/T (4 × 250 mm, sorbent particle size 5 μm), eluent acetonitrile to distilled water. The column was preliminarily calibrated with phenylacetic acid. The enzymatic reactions were performed with immobilized PcAm (activity 10^3 U/g).

Synthesis of (α-aminobenzyl)(hydroxymethyl)-phosphinic acid (I). *a*. To a solution of 20.5 g of (hydroxymethyl)phosphonous acid and 12.6 g of acetamide in 20 ml of acetic anhydride, 21.7 g of benzaldehyde was added. The resulting mixture was slowly heated with stirring to 70°C, after which the mixture spontaneously warmed up to 120°C. The boiling reaction mixture was stirred for 5 h. The ^{31}P NMR spectrum of the reaction mixture showed a singlet at 45 ppm assignable to [α-(acetylamino)benzyl]phosphinic acid (**III**). After cooling, the reaction mixture was evaporated in a vacuum. The oily residue was diluted with 150 ml of conc. HCl, and the mixture was refluxed for 7 h, cooled, concentrated to ca. 70 ml, and washed with benzene (2 × 20 ml) and ether (2 × 20 ml). The aqueous phase was evaporated in a vacuum, and the residue was subjected to cation-exchange chromatography (eluent H_2O–0.5 N HCl). Fractions with a positive ninhydrin test were collected and evaporated in a vacuum. The residue was dissolved in aqueous ethanol and treated with excess propylene oxide to obtain 17.8 g of acid **I**. Yield 42.1% [per (hydroxymethyl)phosphonous acid], mp 228–230°C. Additional crystallization from aqueous ethanol gave an analytically pure sample of acid **I**. Yield 15.8 g (37%), mp 235– 238°C. ^1H NMR spectrum (D_2O + DCl), δ, ppm: 3.37 d (2H, J_{HP} 6.5 Hz); 4.25 d (1H, J_{HP} 11.0 Hz); 7.38 m (5H). ^{31}P NMR spectrum (D_2O + DCl), δ_P, ppm: 34.5. Found, %: C 43.75, 43.63; H 6.47, 6.48; P 14.19, 14.16. $C_8H_{12}NO_3P \cdot H_2O$. Calculated, %: C 43.84; H 6.44; P 14.31.

*b. 1. Synthesis of [α-(Benzylamino)benzyl]-(hydroxymethyl)phosphinic acid (**IV**).* A solution of 2.1 g of (hydroxymethyl)phosphonous acid and 2.4 ml of benzylamine in 8 ml of dry toluene was heated with vigorous stirring to 50°C, and 2.2 ml of benzaldehyde was added slowly to the solution over the course of 15 min. The reaction mixture warmed up to 80°C and was stirred at that temperature for 2 h, after which it was evaporated in a vacuum. The residue was treated with 20 ml of 2 N HCl, insoluble particles were filtered off, and the filtrate was evaporated in a vacuum. The residue was dissolved in aqueous ethanol (~1:5) and treated with excess propylene oxide. Additional crystallization from aqueous ethanol gave 4.5 g of acid **IV**. Yield 70% [per (hydroxymethyl)phosphonous acid]. ^1H NMR spectrum (D_2O + DCl), δ, ppm: 3.22 d.d (2H, CH_2O, J_{HP} 7.0 Hz, J_{HH} 6.7 Hz); 3.45 d (1H, CH_2Ph, J_{HH} 13.3 Hz), 3.97 d (1H, CHP, J_{HP} 10.7 Hz), 6.70 m (2H, 2Ph), 6.90 m (8H,

2Ph). ^{31}P NMR spectrum (D_2O + DCl), δ_P, ppm: 33.8. Found, %: C 51.94, 52.03; H 7.07, 6.98; P 8.88, 8.76. $C_{15}H_{18}NO_3P \cdot H_2O$. Calculated, %: C 52.17; H 7.01; P 8.97.

*2. Hydrogenation of [α-(benzylamino)benzyl](hydroxymethyl)phosphinic acid (**IV**).* To a solution of 5 g of acid **IV** in 30 ml of ethanol, 1.8 g of ammonium formate and 1 g of 10% Pd/C were added. The resulting mixture was stirred for 15 h at room temperature, the catalyst was removed by filtration through a Celite bed, and the filtrate was evaporated in a vacuum. The residue was dissolved in a minimum of water and passed through a strongly acidic cation-exchange resin to remove ammonium ions. Fractions with a positive ninhydrin test were combined and evaporated in a vacuum. The residue was crystallized from aqueous ethanol to give 1.9 g of acid **I**. Yield 64.5% [per acid **IV**, total yield 45.5% per phosphonous acid **II**]. mp 233–235°C. The ^{1}H and ^{31}P NMR spectra (D_2O + DCl) were identical to those of the amino acid prepared by procedure *a*.

Synthesis of (*R*,*S*)-(hydroxymethyl)[α-[(phenylacetyl)amino]benzyl]phosphinic acid (V). To a vigorously stirred solution of 17.8 g of racemic aminophosphinic acid **I** in 70 ml of water, 14.0 ml of phenylacetyl chloride was added dropwise at 0–5°C and pH 9 maintained with 50% aqueous KOH. The reaction mixture was then acidified to pH 5 and extracted with ether (2 × 30 ml). The aqueous phase was acidified to pH 2 and extracted with ethyl acetate (3 × 50 ml). The extract was dried over $MgSO_4$ and evaporated in a vacuum. The residue was crystallized from ether and recrystallized from a hexane–acetone mixture to isolate an analytically pure acid **V**. Yield 17.8 g [63% per (*R*,*S*)-**I**]. mp 115–117°C. ^{1}H NMR spectrum (CD_3OD), δ, ppm: 3.66 m (4H, C*H*$_2$OH + C*H*$_2$Ph); 5.5O d (1H, CH, J_{HP} 14 Hz), 7.30 m (10H, 2 Ph). ^{31}P NMR spectrum (CD_3OD), δ_P, ppm: 41.6. Found,%: C 59.28, 59.03; H 5.66, 5.78; P 9.31, 9.46. $C_{16}H_{18}NO_4P \cdot H_2O$. Calculated, %: C 59.35, H 5.76, P 9.57.

Enzymatic synthesis of (*R*)-I and (*S*)-I. Racemic acid **IV** was dissolved in 80 ml of water at pH 7.5 (maintained with 1 N KOH), and preliminary prepared PcAm (~5 g) was added to the solution. The resulting mixture was stirred at room temperature (20–25°C). Reaction progress was controlled by means HPLC by the amount of phenylacetic acid in the solution. After the reaction was complete, the enzyme was filtered off, and the filtrate was extracted with ether (2 × 20 ml), acidified to pH 5, and extracted again with ether (2 × 20 ml). The aqueous layer was concentrated in a vacuum to ca. 20 ml and then passed through a cation-exchange column (H^+) (3 × 30 cm), eluent water.

1. Fractions with a negative ninhydrin test were collected and evaporated in a vacuum. The residue was crystallized from ether to give 2.9 g (61.7%) of acid (*R*)-**V**, mp 131-134°C. The ^{1}H and ^{31}P NMR spectra of the sample were identical to those of (*R*,*S*)-**V**. Found, %: C 59.36, 59.13; H 5.64, 5.67; P 9.38, 9.35. $C_{16}H_{18}NO_4P \cdot 0.25H_2O$. Calculated, %: C 59.35, H 5.76, P 9.57. $[\alpha]^{25}_{546}$ –19.3° (*c* 1.0, EtOH).

A mixture of 1.0 g of acid (*R*)-**V** and 10 μl of 6 N HCl was refluxed for 10 h and then evaporated. The residue was partitioned between 20 ml of water and 5 ml of ether. The aqueous phase was washed with ether (2 × 10 ml) and evaporated in a vacuum. The residue was treated with excess propylene oxide in aqueous ethanol (~1 : 7) to obtain 0.53 g of acid (*R*)-**I**. Yield 84.0% per (*R*)-**V** and 51.6% per (*R*,*S*)-**V**. mp 190–195°C (softening) and 233–235°C (melting with decomposition). The ^{1}H and ^{31}P NMR spectra of the sample were identical to those of (*R*,*S*)-**I**. Found, %: C 47.37; 47.65; H 5.95, 6.22; P 15.12, 15.31. $C_8H_{12}NO_3P$. Calculated,%: C 47.77; H 6.01; P 15.40. $[\alpha]^{25}_{546}$ +4.1° (*c* 1.0, 6N HCl); $[\alpha]^{25}_{546}$ +8.2° (*c* 1.0, H_2O).

2. Fractions with a positive ninhydrin test were collected (eluent 0.5 N HCl) and evaporated. The residue was treated with excess propylene oxide in aqueous ethanol (~1 : 7) to obtain 1.7 g of acid (*S*)-**I**. Yield 57.4% per (*R*,*S*)-**V**. mp 170–174°C (softening), 221–224°C (melting with decomposition). ^{1}H and ^{31}P NMR spectra in D_2O were identical to those of (*R*,*S*)-**I**. Found, %: C 43.59, 43.75; H 6.39, 6.27; P 13.97, 13.81. $C_8H_{12}NO_3P \cdot H_2O$. Calculated, %: C 43.84, H 6.44, P 14.13. $[\alpha]^{25}_{546}$ –4.0° (*c* 1.0 6N HCl); $[\alpha]^{25}_{546}$ –8.3 (*c* 1.0, H_2O).

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REFERENCES

1. Collinsova, M. and Jiracek, J., *Curr. Med. Chem.*, 2000, vol. 7, no. 6, p. 629.
2. Cherkasov, R.A. and Galkin, V.I., *Usp. Khim.*, 1998, vol. 67, no. 10, p. 940.

3. Baylis, E.K., *Tetrahedron Lett.*, 1995, vol. 36, no. 51, p. 9389.
4. Gancarz, R., Gancarz, I., and Walkowjak, U., *Phosphorus, Sulfur, Silicon Relat. Elem.*, 1995, vol. 104, no. 1, p. 45.
5. Hammerschmidt, F. and Hanbauer, M., *J. Org. Chem.*, 2000, vol. 65, no. 19, p. 6121.
6. Roos, G.H. and Balasubramaniam, S., *Synth. Commun.*, 1998, vol. 28, no. 20, p. 3877.
7. Boyd, E.A., Chan, W.C., and Loh, V.M., *Tetrahedron Lett.*, 1996, vol. 37, no. 10, p. 1647.
8. Mucha, A., Kafarski, P., Plenat, F., and Cristau, H.-J., *Phosphorus, Sulfur, Silicon Relat. Elem.*, 1995, vol. 105, nos. 1–4, p. 87.
9. Kafarski, P., Lejczak, B., Tyka, R., Koba, L., Pliszczak, E., and Wieczonek, P., *J. Plant Growth Regul.*, 1995, vol. 14, no. 4, p. 199.
10. Zon, J., Amrhein, N., and Gancarz, R., *Phytochemistry*, 2002, vol. 59, no. 1, p. 9.
11. Oleksyszyn, J. and Gruszecka, E., *Tetrahedron Lett.*, 1981, vol. 22, no. 36, p. 3537.
12. Yiotakis, A., Vassiliou, S., Jiracek, J., and Dive, V., *J. Org. Chem.*, 1996, vol. 61, no. 19, p. 6601.
13. Miller, D.J., Hammond, S.M., Anderluzzi, D., and Bugg, T.D.H., *J. Chem. Soc., Perkin Trans. 1*, 1998, no. 1. p. 131.
14. Solodenko, V.A., Kasheva, N.A., Kukhar, V.P., Kozlova, E.V., Mironenko, D.A., and Svedas, V.-K., *Tetrahedron*, 1991, vol. 47, no. 24, p. 3989.
15. Rozhko, L.F., Klochkov, S.G., Ragulin, V.V., and Tsvetkov, E.N., *Zh. Obshch. Khim.*, 1999, vol. 69, no. 7, p. 1134.
16. Schotten, C., *Ber.*, 1884, vol. 17, p. 2544; Baumann, E., *Ber.*, 1886, vol. 19, p. 3218.
17. Smith, A.B., Yager, K.M., and Taylor, C.M., *J. Am. Chem. Soc.*, 1995, vol. 117, no. 44, p. 10879.
18. Hamilton, R., Walker, B., and Walker, B.J., *Tetrahedron Lett.*, 1995, vol. 36, no. 25, p. 4451.
19. Mikolajszyk, M., Lyzwa, P., and Drabowicz, J., *Tetrahedron: Asymmetry*, 1997, vol. 8, no. 24, p. 3991.
20. Glowiak, T., Sawka-Dobrowolska, W., Kovalik, J., Mastalerz, P., Soroka, M., and Zon, J., *Tetrahedron Lett.*, 1977, vol. 45, no. 13, p. 3965.
21. Uhing, E.H., Rattenbury, K., and Toy, A.D.F., *J. Am. Chem. Soc.*, 1961, vol. 83, no. 5, p. 2299.