Synthesis and fungicidal activity of aminoalkylthiophosphonates

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Two methods for the synthesis of 1-aminoalkylthiophosphonic acids were developed, and these compounds were found to exhibit a high biological activity.

The well-known physiologically active organophosphorus analogues of amino acids mainly belong to structurally different phosphinic or phosphonic acids **1** and **2**, respectively, which exert different effects on amino acid metabolism. Qualitatively, the P(O)(OH)H group in place of the carboxyl group of an amino acid has no effect on the affinity of aminoalkylphosphinates **1** to enzymes involved in amino acid metabolism and on the capability of these amino acid isosteres for substrate conversions. It also does not prevent their permeation through the cell walls of microorganisms.[1] In aminoalkylphosphonates **2**, the dibasic $P(O)(OH)_2$ group is dramatically different from the carboxyl group in size, geometry, and acidity. These evident structural differences between aminocarboxylic acids and aminoalkylphosphonates **2** are unfavourable for their transport into microbial cells.[2,3] At the same time, these structure peculiarities allowed us to consider aminoalkylphosphonates **2** as stable analogues of labile transition states of substrates in amino acid conversions and to develop new efficient inhibitors for amino acid metabolism enzymes on this basis.[4,5] Thus, the modification of only the phosphorus-containing moiety affects the main biochemical characteristics of phosphorus-containing amino acid analogues by modulating their biochemical activity.

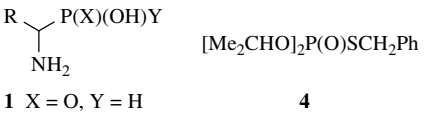
Here, we describe the synthesis of 1-aminoalkylthiophosphonic acids **3** and exemplify the biological activity of this new class of compounds, whose thiophosphonate fragment is more bulky than the phosphonate group.

Alkylthiophosphonates are stable only as salts, which are prepared by the hydrolysis of corresponding acid dichlorides.[6] In the series of aminoalkylthiophosphonates **3**, O,O-diesters are known. They are synthesised by the reactions of carbonyl compounds with NH_3 and $P(S)(OR)_2H$; their hydrolysis under mild or more severe conditions leads to O-monoesters or phosphonic acids, respectively.[6] The reactions of the simplest alkylphosphinates with melted sulfur for several hours afforded corresponding phosphonic acid anhydrides and trace thiophosphonates, which can be conveniently synthesised based on silylated alkylphosphinates.[7]

We developed a general method for the synthesis of thiophosphonates **3** from available acids **1**, whose amino group is protected by protonation. The reaction with sulfur was performed on short-time heating or incubation in DMF under normal conditions (Scheme 1, A).†

Another procedure, which afforded similar yields, consists in the silylation of acids **1** followed by the addition of sulfur and hydrolytic deblocking of the reaction product (Scheme 1, B),‡ analogously to the published procedure.[7]

Thiophosphonates **3** are stable in storage in a matter of months. They were converted into well-known phosphonates **2** under the action of acids, alkalis, heavy metal ions and oxidizing agents. They have a thione structure and undergo alkylation to form thioesters. Under model conditions, they can form azomethines with pyridoxal 5′-phosphate, the coenzyme of pyridoxal enzymes. In this respect, they are not qualitatively different from acids **1** and **2**. However, unlike phosphinates **1**, the thiophosphonate analogues of substrate amino acids cannot be cleaved by pyridoxal lyases, and they do not inhibit these enzymes.



1 X = O, Y = H
2 X = O, Y = OH
3 X = S, Y = OH

4

Scheme 1

† A solution of 0.05 mol of acid **1** in 30 cm^3 of 2 M HBr was vacuum evaporated to dryness; 50 cm^3 of isopropanol were added to the residue, and the solvent was distilled in a vacuum. This procedure was repeated two times; next, 100 cm^3 of DMF were added to the residue and 50 cm^3 of the solvent were distilled in a vacuum. 3.2 g (0.1 mol) of powdered sulfur were added to the residue, and the mixture was slowly heated to 125 °C in a nitrogen atmosphere with stirring and allowed to stand at this temperature for 10 min or the mixture was stirred at 20 °C for 5–10 days until the disappearance of parent acid **1**. Next, the mixture was filtered, and the filtrate was vacuum evaporated to dryness. Water (20 cm^3) was added to the residue, and the contents were neutralised with 10% NH_4OH to pH 6. After filtration, the product was separated on a column (22×3 cm) packed with Dowex 50×8 resin (H^+ form), which was equilibrated with 15% aqueous isopropanol. The elution was performed with the same solvent. Fractions containing acid **3** were vacuum evaporated to dryness; the residue was dissolved in hot water, and crystallisation was induced by the addition of an alcohol. The melting temperatures were determined in open capillaries and were not corrected. Ascending TLC was performed on Silufol UV_{254} plates (Kavalier, Czech Republic) using the following systems (v/v): Pr^iOH–25% NH_4OH–H_2O, 7:1:2 (A) and MeOH–Pr^iOH–25% NH_4OH–H_2O–$CF_3CO_2NH_4$, 100:40:20:40:1 (B). The spots were visualised using reactions with ninhydrin and ammonium molybdate.

Acid **3a**: yield 68%, mp 202–204 °C (decomp.). R_f 0.17 (A), 0.25 (B). ^{1}H NMR (400 MHz, D_2O) δ: 2.98 (d, 2H, $^2J_{H,P}$ 10.5 Hz). ^{31}P NMR (D_2O) δ: 59.79 (t, 1P, $^2J_{PH}$ 10.5 Hz). Found (%): C, 9.70; H, 4.89; N, 10.90. Calc. for CH_6NO_2PS (%): C, 9.45; H, 4.76; N, 11.02.

Acid **3b**: yield 63%, mp 210–214 °C (decomp.). R_f 0.19 (A), 0.31 (B). ^{1}H NMR (400 MHz, D_2O) δ: 1.25 (dd, 3H, $^3J_{H,H}$ 7.2 Hz, $^3J_{H,P}$ 16.2 Hz), 3.21 (dq, 1H, $^3J_{H,H}$ 7.2 Hz, $^2J_{H,P}$ 10.5 Hz). ^{31}P NMR (400 MHz, D_2O) δ: 65.05 (dq, 1P, $^3J_{PH}$ 16.2 Hz, $^2J_{PH}$ 10.5 Hz). Found (%): C, 17.55; H, 5.93; N, 9.46. Calc. for $C_2H_8NO_2PS$ (%): C, 17.02; H, 5.71; N, 9.92.

Acid **3c**: yield 56%, mp 224–226 °C (decomp.). R_f 0.37 (A). ^{1}H NMR (400 MHz, D_2O) δ: 0.88 and 0.93 (2d, 6H, $^3J_{HH}$ 6.8 Hz), 2.06–2.18 (m, 1H), 2.85 (dd, 1H, $^2J_{HP}$ 11.2 Hz, $^3J_{HH}$ 6.5 Hz). ^{31}P NMR (400 MHz, D_2O) δ: 62.02 (dd, 1P, $^2J_{PH}$ 11.2 Hz, $^3J_{PH}$ 10.2 Hz). Found (%): C, 28.62; H, 7.11; N, 8.08. Calc. for $C_4H_{12}NO_2PS$ (%): C, 28.40; H, 7.15; N, 8.28.

Acid **3d**: yield 54%, mp 222–224 °C (decomp.). R_f 0.40 (A), 0.58 (B). ^{1}H NMR (400 MHz, D_2O) δ: 0.71 and 0.76 (2d, 6H, $^3J_{HH}$ 6 Hz), 1.51–1.57 (m, 3H), 3.10–3.17 (m, 1H). ^{31}P NMR (400 MHz, D_2O) δ: 64.8–65.1 (m, 1P). Found (%): C, 32.75; H, 8.03; N, 7.40. Calc. for $C_5H_{14}NO_2PS$ (%): C, 32.78; H, 7.70; N, 7. 65.

‡ 4.2 cm^3 (0.03 mol) of Et_3N and 3.3 g (0.03 mol) of Me_3SiCl were added to a suspension of 0.01 mol of acid **1** in 15 cm^3 of dry $CHCl_3$ in an atmosphere of N_2 at 20 °C with stirring. After 2 h, 0.5 cm^3 of Et_3N, 0.5 cm^3 of Me_3SiCl and 3.3 g (0.03 mol) of powdered sulfur were added, and the mixture was stirred for 12 h at 20 °C. The mixture was filtered, and 10 cm^3 of methanol were added to the filtrate; after 1 h, the mixture was vacuum evaporated to dryness at 20 °C, and 10 cm^3 of water were added to the residue. The solution was filtered, and acid **3** was isolated from the filtrate as described above.

Acid **3c**, yield 67%, identical to the substance described above.

Table 1 The fungicidal activity of thiophosphonates **3** and corresponding phosphinates **1** (in parentheses) in a minimum medium.

Compound	G	C
3a	1 (50)	1 (250)
3b	5 (5)	0.5 (50)
3c	75 (75)	75 (150)
3d	10 (1)	1 (120)
3e[a]	10 (0.5)	4 (50)
4 Kitazine	40	No data

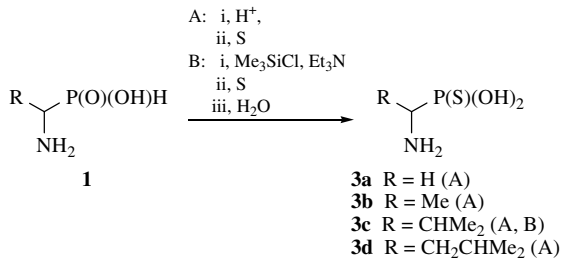
[a]The synthesis of acid **3e** (R = CH_2OH) will be published elsewhere.

The biological activity of thiophosphonates **3** in comparison with phosphinates **1** and phosphonates **2** was tested on phytopathogenic fungi. In the case of a pathogen of rice, fungus *Magnaporthe grisea* (Hebert) Barr, the tests were performed in both a standard agar medium and a minimum medium containing only inorganic salts, glucose, thiamine and biotin in accordance with the published procedure.[8] The fungicidal activity of thiophosphonates **3** was characterised by effective concentrations (EC50, μg cm^{-3}), which inhibited the growth of colonies (G) and germination of conidium (C) by 50%. The results of these studies in a minimum medium are given in Table 1, where EC50 for corresponding phosphinates **1** are given in parentheses.

These data indicate that thiophosphonates **3** exhibit a high fungicidal activity, which implies the capability of penetrating into microbial cells (unlike phosphonates **2**). They selectively inhibit the germination of conidium, that is, the formation of new colonies. In this respect, they are different from phosphinates **1**. The activity of thiophosphonates **3** depends on the structures of both the radical and the phosphorus-containing functional group: O,O-diesters are passive, and O-monoesters are inferior to acids **3**. The effect of thiophosphonates **3** in a standard medium was weaker; this is evidently due to competition with the amino acids of the medium. The fungicidal activity of thiophosphonates **3** can be evaluated by comparing it with the effect of the practically important fungicide Kitazine P **4**.[9]

The *in vitro* growth inhibition of the fungus *Rizoctonia solani* by thiophosphonates **3b–d** (0.003% aqueous solutions) exemplifies the activity towards other phytopathogenic fungi. In the field experiments, thiophosphonate analogues **3c** and **3d** of valine and leucine, respectively, were highly competitive with a reference fungicide in crop capacity control.

Thus, the introduction of a bulky thiophosphonate functional group (the van der Waals radii of O and S atoms are equal to 1.45 and 1.85 Å, respectively) in place of the carboxyl group allowed us to obtain new analogues of amino acids capable of transporting through cell walls and exhibiting high biological activity.



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3a R = H (A)
3b R = Me (A)
3c R = $CHMe_2$ (A, B)
3d R = CH_2CHMe_2 (A)

Scheme 2

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