New synthesis and fungicidal activity of the phosphine analogues of serine and threonine

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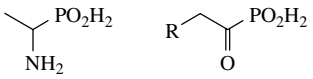
The phosphine analogues of serine and threonine were prepared by the reaction of hydroxyaldehyde oximes with H_3PO_2, and the acid ester of sulfuric acid and the phosphine analogue of serine was synthesised; the high fungicidal activity of these substances was found for the first time.

A target-oriented search for biologically active compounds in the series of aminoalkylphosphinic acids, which is based on studies of the metabolism and enzymatic conversion of these analogues of natural amino acids, has been implemented previously only in isolated instances. Thus, the fungicidal activity of 1-aminoethylphosphinic acid **1** (Scheme 1) towards the phytopathogenic fungus *Magnaporthe grisea* (Hebert) Barr and the inhibition of the biosynthesis of melanin,[1] which plays an important role in pathogenesis, are due to the capability of aminoalkylphosphinates to enzymatic transamination.[2] Similarly to alanine, acid **1** enters the cell and changes into 1-oxoethylphosphinic acid **2**, which inhibits pyruvate dehydrogenase and the formation of acetyl-CoA and thereby blocks the biosynthesis of melanin and fungal growth.[3] In a similar manner, the inhibition of the biosynthesis of anthocyanin (a plant pigment) by compound **1** was explained.[4]



1 **2** R = H, **3** R = Me

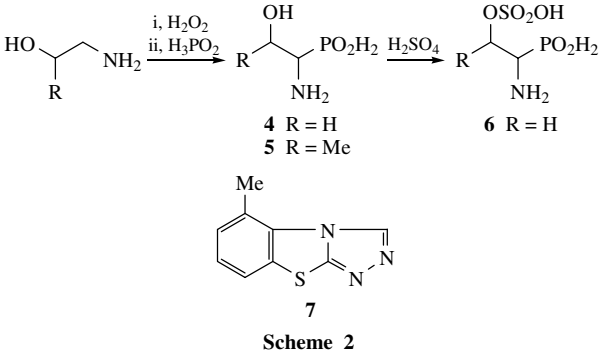
Scheme 1

It was found that aminoalkylphosphinates can undergo important transformations, namely, degradation under the action of pyridoxal β- and γ-lyases.[5,6] The products of these reactions are the phosphine analogues of pyruvate **2** and α-ketobutyrate **3**, which belong to dehydrogenase inhibitors and are incapable to penetrate through the cell walls of microorganisms.[3] The biological importance of such transformations remained unclear and depended on the natural occurrence of the corresponding enzymes.

This work was devoted to the synthesis and characterization of the fungicidal activity of the analogues of serine **4** and threonine **5**. These compounds were chosen as potential biologically active compounds because deamination to pyruvate and α-ketobutyrate under the action of corresponding dehydratases is an important pathway in the catabolism of serine and threonine in microorganisms.[7,8]

The well-known syntheses of acids **4** and **5** include the preparation of *O*-benzyl ethers of glycolic and β-hydroxypropionic aldehydes, their conversion into *N*-benzhydrilidene derivatives, the reaction with H_3PO_2 and the removal of protective groups.[9] We found that the analogues of α-amino-β-hydroxy acids can be prepared by the direct interaction of hydroxyaldehyde oximes with H_3PO_2 according to a known reaction.[10] The procedure can be significantly simplified by the use of readily available alkanolamines with the subsequent oxidation with H_2O_2 to oximes[11] (Scheme 2).

Acid **4**[†] became available because of a simple synthesis; however, threonine analogue **5** was obtained in a lower yield under the same conditions.[‡] The synthesis of previously unknown acid ester of sulfuric acid and the phosphine analogue of serine



4 R = H
5 R = Me
6 R = H
7

Scheme 2

6 seemed to be a nontrivial problem because of the absence of appropriate methods for the protection of the phosphine moiety.[12] This problem was solved in the simplest manner by the reaction of serine analogue **4** with H_2SO_4.[§]

Acid **4** was expected to exhibit fungicidal activity and to inhibit the synthesis of melanin because it can penetrate into the cell similarly to other aminophosphinates and undergo α,β-elimination under the action of serine dehydratase with the conversion into pyruvate dehydrogenase inhibitor **2**. In the case of threonine analogue **5**, the effective inhibition of melaninogenesis seemed less probable because its cleavage with threonine dehydratase resulted in acid **3**, which weakly inhibits pyruvate dehydrogenase, as distinct from pyruvate analogue **2**.[13] At the same time, threonine analogue **5** could exhibit fungicidal activity because acid **3** is an evident competitor of

† 25 cm^3 of a 30% H_2O_2 solution were added to a solution of 6.1 g (0.1 mol) of ethanolamine and 0.5 g of Na_2WO_4 in 15 cm^3 of water, adjusted to pH 3 with 20% HCl, with stirring for 1.5 h at 5–10 °C. Next, the mixture was additionally stirred for 3 h, evaporated in a vacuum to 15 cm^3, saturated with NaCl and extracted with ethyl acetate (5×20 cm^3). The joined extracts were filtered through $MgSO_4$, vacuum evaporated to 20 cm^3, and added to a solution of 13 g (0.2 mol) of anhydrous H_3PO_2 in 50 cm^3 of MeOH under reflux in a nitrogen atmosphere with stirring for 30 min. The mixture was additionally refluxed for 30 min, cooled and evaporated in a vacuum. The residue was dissolved in water (15 cm^3), and the product was separated on a 100 cm^3 column with Dowex 50×8 resin (H^+ form); elution with 15% aqueous isopropanol. Fractions containing compound **4** were vacuum evaporated to dryness; the residue was vacuum dried over P_2O_5 to obtain compound **4** (3.1 g, 25%); mp 205–207 °C (decomp.) [lit.,[9] 210 °C (decomp.)]. R_f 0.41 (Pr^iOH–25% NH_4OH–H_2O, 7:1:2, system A). ^{1}H NMR (400 MHz, D_2O) δ: 3.07–3.14 (m, 1H, CH), 3.68–3.91 (m, 2H, OCH_2), 6.91 (d, 1H, PH, *J* 540 Hz).

‡ Compound **5** (0.35 g, 5%) was prepared from 3.3 g (0.05 mol) of aminoisopropanol in accordance with the above procedure; mp 215–217 °C (decomp.) [lit.,[9] 218–220 °C (decomp.)]. R_f 0.47 (A). ^{1}H NMR (400 MHz, D_2O) δ: 1.18, 1.22 and 1.25 (3d, 3H, diastereomeric MeCH, *J* 6.9 Hz), 2.83–2.88 and 3.03–3.10 (2m, 1H, diastereomeric CH), 3.96–4.01 and 4.14–4.22 (2m, 1H, diastereomeric OCH), 6.80, 6.90 and 6.97 (3d, 1H, diastereomeric PH, *J* 540 Hz).

α-ketobutyrate, a key compound in the biosynthesis of isoleucine and propionyl–CoA. The transformation of serine analogue **4** into ester **6** with a good leaving group at the β-position opened up an additional opportunity to generate pyruvate analogue **2**, namely, under the action of aspartate aminotransferase, analogously to the conversion of the sulfate ester of serine under the action of this enzyme.[14]

The effects of phosphinic acids **4**–**6** on the fungus *M. grisea* were studied in accordance with a procedure described elsewhere.[1] In *in vitro* experiments, to evaluate the effect of amino acids on the activity of analogues, the effects of analogues were studied in both a standard agar medium and in a medium that contained only inorganic salts, glucose, thiamine, and biotin (a minimum medium). The fungicidal activity of phosphinic acids was characterised by effective concentrations (EC50, μg cm^{-3}), which inhibited the growth of colonies (G) and the germination of conidia (C) by 50%. The effect on melaninogenesis (M) was evaluated by the retention of pigmentation (+) or decolouration of mycelium (–). The results of these studies on growing the fungus *M. grisea* in a minimum medium are given below.

Analogue	G	C	M
4	1.0	50	(–)
5	0.2	4	(±)
6	1.0	50	(–)

As follows from these data, phosphinic acids **4** and **6** effectively inhibit the growth of mycelium and cause its full decolouration in the same concentrations, which are comparable to the activity of an optimum inhibitor of melaninogenesis (EC50 0.1–1 μg cm^{-3}). Tricyclazol **7**, which is one of the best fungicides against rice diseases, in the same doses blocked the formation of melanin; however, in this case, the inhibition of mycelium growth and the germination of conidia occurred at 400 and 200 μg cm^{-3}, respectively. Threonine analogue **5** is characterised by high fungitoxicity, but it weakly affects the pigmentation of mycelium. This is a new type of fungicidal activity, which is not related to the inhibition of the biosynthesis of melanin. In a standard medium, phosphinic acids **4**–**6** at concentrations of 50–100 μg cm^{-3} inhibit the growth of mycelium, whereas alanine analogue **1** is practically inactive (EC50 > 1000 100 μg cm^{-3}); the effect of Tricyclazol **7** does not depend on the composition of the medium. A decrease in activity and the retention of mycelium pigmentation in this case are indicative of competitive relations between the analogues and natural amino acids.

A study of the fungicidal activity of serine analogue **4** demonstrated that, unlike Tricyclazol, it also acts against other phytopathogenic fungi. In a concentration of 10 μg cm^{-3}, it inhibited the growth of the fungus *Plasmopara viticola* (Bevl. Et de-Toni). As a 0.05% aqueous solution, it completely inhibited the development of septoriosis in wheat leaves. Serine analogue **4** did not exhibit phytotoxicity, which is typical of alanine analogue **1**,[9] in preliminary experiments. This is likely due to different metabolic pathways of different precursors of the same melaninogenesis inhibitor **2**.

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§ Compound **4** (1.25 g 0.01 mol) was added to 2 cm^3 of 95% H_2SO_4 at 0 °C with intense stirring; the mixture was additionally stirred for 60 min at 20 °C and then poured into 50 cm^3 of cold (–10 °C) absolute diethyl ether with stirring. The ether was decanted, and the residue was washed triply with cold diethyl ether and then dissolved in 25 cm^3 of ice H_2O and a saturated aqueous $Ba(OH)_2$ solution was added until the completion of precipitation. The mixture was filtered; the filtrate was vacuum evaporated to 5 cm^3, and the product was separated on a 100 cm^3 column with Dowex 50×8 resin (H^+ form); elution with 15% aqueous isopropanol. Fractions containing compound **6** were vacuum evaporated; the oily residue was dissolved in 5 cm^3 of MeOH, and the solution was neutralised with an NH_3 solution in MeOH (saturated at 20 °C) on cooling. The mixture was vacuum evaporated; the residue was vacuum dried over P_2O_5. Ammonium salt **6** (1.44 g, 65%) was obtained, mp 203–204 °C (decomp.). R_f 0.35 (A). ^{1}H NMR (400 MHz, D_2O) δ: 3.32–3.38 (m, 1H, CH), 4.16–4.35 (m, 2H, OCH_2), 6.95 (d, 1H, PH, J 548 Hz). ^{31}P NMR (400 MHz, D_2O) δ: 13.74 (dddd, 1P, PH, $^1J_{PH}$ 549.3 Hz, $^2J_{PH}$ 9.65 Hz, $^3J_{PH_A}$ 9.96 Hz, $^3J_{PH_B}$ 3.4 Hz).

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