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The Preparation of 2-Guanidinoethyl Phosphate

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During the isolation of lombricine from *Lumbricus terrestris* L. (Pant, 1959), it was observed that when the concentrated ammoniacal eluate from the $C_{50}D$ cation column was kept in the refrigerator for 4–5 days, a white crystalline solid separated out. This compound on being purified was identified chromatographically as guanidinoethyl phosphate, which is one of the products of hydrolysis of lombricine (Van Thoai & Robin, 1954). Our repeated attempts to prepare this compound by the only available method described by Van Thoai & Robin (1954) proved unsuccessful. However, the recent synthesis of monophosphotaurocyamine by Morrison, Ennor & Griffiths (1958) suggested to us the possibility of its synthesis by a similar procedure.

This paper deals with the details of the preparation of guanidinoethyl phosphate and the study of some of its properties.

EXPERIMENTAL

Paper chromatography. Paper partition chromatography of Consden, Gordon & Martin (1944) was employed. Whatman no. 1 filter-paper sheets were used. The spots were developed at room temperature (22°) for about 7–8 hr. and the solvent was allowed to ascend a distance of about 23–25 cm. The operation was conducted in an air-tight glass-aquarium tank fitted with a lid. The atmosphere of the tank was kept saturated with the vapours of the solvent.

Spray reagents. The following were used: ninhydrin solution (0·1%, w/v) in butanol; Sakaguchi reagents (Pant, 1959) and molybdate reagents (Hanes & Isherwood, 1949).

Preparation of 2-aminoethanol 1-phosphate. This ester was prepared by treating phosphoryl chloride with aq. 2-aminoethanol as described by Outhouse (1937). To phosphoryl chloride (15 ml.) placed in a 500 ml. (Büchner) flask and cooled by immersion in a salt–ice mixture (−5°) an ice-cold mixture of 2-aminoethanol (10 ml., 1 mol.) and water (5 ml., 2 mol.) was added dropwise from a burette. The reaction proceeded with evolution of heat and copious fumes of HCl. When the reaction was complete, the flask was removed from the cold bath and evacuated with a water suction pump at room temperature for about 4 hr. until almost all the HCl formed had been removed. Water (500 ml.) was then added to the reaction mixture with stirring until the gummy mass formed was completely dissolved. The solution was made alkaline to pH 10·0 by addition of hot saturated baryta solution (approx. 400 ml.). The precipitated barium phosphate was centrifuged, washed twice with water (50 ml.), the washings were combined with the first supernatant and the volume was reduced to 50 ml. on a water bath. The solution was cooled and ethanol (80 ml.) was added in small quantities to precipitate the barium 2-aminoethanol 1-phosphate; it was then left at 0° for 2–3 hr. The white crystalline solid (8 g.) was centrifuged off and twice washed with ethanol (30 ml.) and ether and dried. The dry barium salt was recrystallized from water with ethanol and dried *in vacuo*. Yield, 7·5 g.

In order to obtain 2-aminoethanol 1-phosphate, the barium salt was dissolved in water (80 ml.) and H_2SO_4 (60 ml., 0·5 N) was added to it until all the barium was completely precipitated as barium sulphate. The precipitate was centrifuged off, twice washed with water (25 ml.), the washings were combined with the original supernatant and the solution was concentrated on a water bath to 50 ml. The concentrate was filtered, made turbid by adding methanol (45 ml.) and left in the refrigerator overnight.

The crystals were filtered off, washed with methanol and ether and dried *in vacuo*. Yield, 4·5 g.; m.p. 243–244° uncorr. [Outhouse (1937), m.p. 244°; Clarke, Datta & Rabin (1955), m.p. (corr.) 243·5–244·5°; 243·0–244°]. The material on analysis gave P, 21·97; N, 9·81. $C_2H_8O_4NP$ requires N, 9·93; P, 21·99%. Chromatography in butanol–acetic acid–water (40:10:50, by vol.) gave only one ninhydrin-positive spot (R_F 0·13).

Guanylation of 2-aminoethanol 1-phosphate and synthesis of 2-guanidinoethyl phosphate. 2-Aminoethanol 1-phosphate was guanylated by *S*-methyl*iso*thiourea in an alkaline medium as described by Schütte (1943) for the preparation of guanidines. Thus 2-aminoethanol 1-phosphate (2 g.) was taken up in aq. NH_3 soln. (sp.gr. 0·88) in a 250 ml. flask and powdered *S*-methyl*iso*thiourea (2 g.) was added to it in small quantities at intervals of 30 min. with vigorous shaking. The reactants went into solution with the evolution of methanethiol. The reaction mixture was left at room temperature for 24 hr. in a fume cupboard and then distilled *in vacuo*. The syrupy mass left in the distillation flask was taken up in water (5 ml.) and filtered. Ethanol (20 ml.) was added to precipitate the guanylated derivative formed and the solution was kept at 0° for 2–3 hr. The white crystalline material was filtered off, washed with ethanol and ether and dried. The guanidine derivative was then purified by repeated crystallization with methanol from water and dried *in vacuo* over P_2O_5. Yield, 3 g. The m.p. was very high, the material remaining solid up to 290°.

The compound obtained gave a single spot on the paper chromatogram in all the solvents tested (1–8, Table 1) and did not reveal any ninhydrin-reacting spot. The spot gave a positive reaction with Sakaguchi reagents and showed the presence of phosphate on spraying with the molybdate reagents.

On hydrolysis with H_2SO_4 (6N, 100–110°) the compound gave guanidinoethanol and inorganic phosphate, as shown by paper chromatography. The compound, when run on a chromatogram together with a sample of hydrolysed lobmricine solution, revealed a spot on spraying with the Sakaguchi reagents, which coincided with the position of guanidinoethyl phosphate of lombricine hydrolysate and gave the same R_F, thereby indicating its identity.

On analysis the material gave C, 19·56; H, 5·52; N, 22·86; P, 16·94. $C_3H_{10}O_4N_3P$ requires C, 19·67; H, 5·46; N, 22·95; P, 17·21%.

Table 1. *R_F values of 2-guanidinoethyl phosphate developed with different solvent systems*

Except for solvent 1, the proportions of the components are by volume. In solvent 1, the atmosphere of the chromatographic tank was kept saturated by means of a beaker containing aq. NH_3 soln. (sp.gr. 0·88).

Composition of solvent	R_F
1 Phenol–NH_3 (80% (w/v); NH_3 atm.)	0·47
2 Butanol–acetic acid–water (40:10:50)	0·13
3 Butanol–water–acetic acid (73:17:10)	0·05
4 Pyridine–*iso*pentanol–water–acetic acid (80:40:40:10)	0·19
5 Pyridine–*iso*pentanol–water (80:40:70)	0·22
6 Pyridine–*iso*pentanol–water–aq. 20% (v/v) NH_3 soln. (80:40:40:10)	0·05
7 Phenol saturated with water	0·49
8 Methanol–water–acetic acid (80:20:10)	0·51

One of us (Pant, 1959) has been able to phosphorylate this compound enzymically by employing an acetone-dried powder of *Lumbricus terrestris* L., as well as by using an homogenate of the worms in the presence of adenosine triphosphate.

DISCUSSION

The search for a method to prepare 2-guanidinoethyl phosphate led us to the only method previously described (Van Thoai & Robin, 1954). According to these authors, a suspension of guanidinoethanol in dry pyridine is added in small quantities to phosphoryl oxychloride in dry pyridine kept mechanically stirred and maintained at −5°. Calcium chloride is then added to the reaction mixture followed by calcium oxide to bring the pH to 8–9. The calcium phosphate is reported to precipitate and from the filtrate the guanidyl phosphate ester is isolated by precipitating with ethanol; the calcium is later removed as calcium oxalate by the addition of oxalic acid. However, the present workers, on repeating the above exactly and using the same quantities, encountered certain difficulties. In the first place, although the reaction mixture was stirred for over 2 hr. the guanidinoethanol did not go into solution. Secondly, it was not possible to adjust the pH of the non-aqueous pyridine medium to 8·0 or 9·0. On the other hand, the synthesis of guanidinoethyl phosphate can easily be achieved by the new method described here. The operations involved are simple and the yield obtained is 75% of the theoretical.

SUMMARY

1. A new method has been described for the preparation of 2-guanidinoethyl phosphate by guanylation of 2-aminoethanol phosphate by *S*-methyl*iso*thiourea. Some of the properties of this compound have been reported.

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