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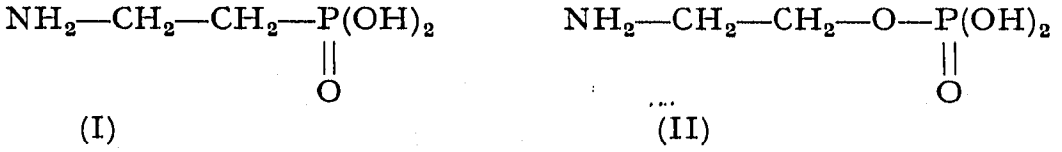
The separation of ciliatine and phosphorylethanolamine

In the last decade, ciliatine or 2-aminoethylphosphonic acid (I) has been detected in the lipid and protein fraction of a great variety of invertebrates[1]. Its structure and its chromatographic properties are similar to phosphorylethanolamine (II) which has also been found in the lipids of some invertebrates[2,3].



(I) (II)

Ciliatine may be readily distinguished from phosphorylethanolamine by its resistance towards drastic hydrolysis with hydrochloric acid, since phosphorylethanolamine is completely hydrolysed into ethanolamine and phosphoric acid on prolonged hydrolysis whereas ciliatine remains unchanged. It is still important however to have a genuine separation procedure which allows the identification and determination of ciliatine and phosphorylethanolamine in a mixture of both these compounds.

Recently NEUZIL *et al.*[4] published a method for the separation of ciliatine and phosphorylethanolamine on ion-exchange paper; this method is however unsuitable for quantitative work.

The present paper describes the separation of these two compounds on a column of the ion-exchange resin Amberlite CG 120 at a pH value of 2.50 and a temperature of 50°. After separation the compounds are estimated by means of their reaction with ninhydrin.

Experimental

The ion-exchange resin, Amberlite CG 120 type 2, was packed to a height of 120 cm in a chromatography column (125 × 0.9 cm) which was jacketed in order to permit temperature control with water. The eluting buffer was prepared by dissolving 105 g of citric acid, 41 g of sodium hydroxide and 52 ml of concentrated hydrochloric acid in 5 l of water; the pH of the buffer was adjusted to the required value with dilute hydrochloric acid or sodium hydroxide.

Ciliatine was obtained from Prof. A. F. ISBELL of Texas A. and M. University and a solution containing 580 μg ciliatine per ml of citrate buffer was prepared. Phosphorylethanolamine was purchased from L. Light and Co. Ltd., Colnbrook, Great Britain and a solution containing 248 μg of phosphorylethanolamine per ml of citrate buffer was prepared.

For the separation experiments 0.5 ml of each of the solutions *i.e.* 290 μg ciliatine and 124 μg phosphorylethanolamine were mixed and carefully placed on the column. The substances were eluted from the column with the citrate buffer and 1-ml fractions were collected with an automatic fraction collector. Ciliatine and phosphorylethanolamine were assayed in the tubes by reaction with ninhydrin in a boiling water bath according to MOORE AND STEIN[5]. After dilution with 10 ml of water the absorbance was read at 565 nm and plotted against tube number. Quanti-

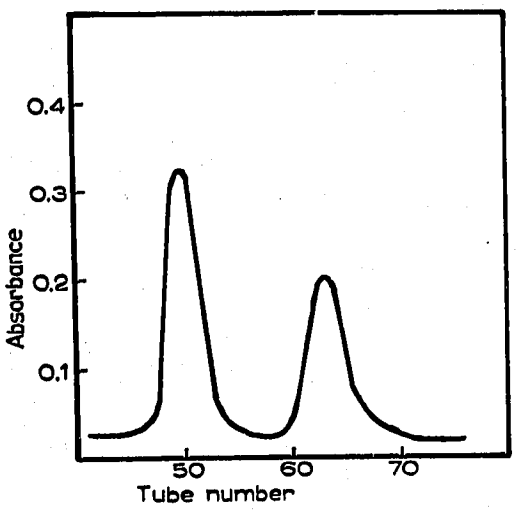


Fig. 1. Separation of 124 μg phosphorylethanolamine and 290 μg ciliatine on the ion-exchange resin Amberlite CG 120 at 50° with a citrate buffer at pH 2.50.

tative estimation of ciliatine in lipid hydrolysates was achieved by comparing the peak area with that obtained from the standard solution.

The extraction and the hydrolysis of lipids have been described elsewhere[6].

Results and discussion

Fig. 1 shows the separation of 124 μg of phosphorylethanolamine and 290 μg of ciliatine on the ion-exchange resin at a pH value of 2.50. Tests with phosphorylethanolamine or ciliatine alone indicated that the first peak is due to phosphorylethanolamine and the second due to ciliatine. Previous work[6] had shown that phosphorylethanolamine and ciliatine could only be partially separated on the ion-exchange resin using a citrate buffer of pH 3.25 (*cf.* ref. 2); at this pH the compounds form a double peak, phosphorylethanolamine having a maximum at tube number 48 and ciliatine at tube number 52. By lowering the pH of the eluting buffer to 2.50 the ciliatine peak shifted considerably, its maximum now being at tube 63, whereas the shift of the phosphorylethanolamine peak was negligible; thus the separation at pH 2.50 was complete as is clearly shown in Fig. 1. This characteristic shift may therefore be regarded—as was done in the present paper—as additional evidence for the presence of ciliatine in hydrolysates.

By means of the above-mentioned procedure we were able to detect and

TABLE I

CILIATINE IN THE LIPIDS OF A MARINE CRAB (*Cyclograpsus punctatus*) AND THE GARDEN SNAIL (*Helix aspersa*)

Source	*Percentage ciliatine in lipids*
Marine crab body	0.10
Marine crab viscera	0.08
Snail body	0.34
Snail viscera	0.19

estimate ciliatine in the lipids of a marine crab (*Cyclograpsus punctatus*) and the garden snail (*Helix aspersa*). The results of this work are shown in Table I and indicate that the body and viscera lipids of the crab and the snail contain small amounts of ciliatine.

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