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Separation of Planktonic Algal Pigments by Thin Layer Chromatography

SIR: In the course of an investigation on planktonic pigments, a rapid method for separation of small quantities of these pigments was needed. A search of the literature revealed several papers on the application of column and paper chromatography (2, 4-8) for the separation of plastid pigments. This paper describes the separation of the chloroplast pigments of algae in thin layers of powdered cellulose. The separations are better than those obtained in thin layers of other adsorbents (1, 3). Moreover, alteration, as indicated by the formation of pheophytins, is at a minimum in the cellulose. It was therefore decided to investigate the separation of these pigments by thin layer chromatography. During the course of this investigation two papers (1, 3) were published that use thin layer procedures for separating plastid pigments. The method reported below was superior to either of the reported methods for thin layer separation of plastid pigments.

EXPERIMENTAL

Separation of Plastid Pigments.

A pure culture of *Scenedesmus quadricauda* was centrifuged, water decanted, and suspended in a 150-ml. mixture of methanol and petroleum ether (3 parts methanol to 1 part P-ether). Extraction was complete in 15 minutes. The extract was filtered through glasswool into a separatory funnel, 500 ml. of 10% sodium chloride solution was added, the solution was mixed by gentle swirling, and the aqueous layer was removed. The deep green petroleum ether solution was washed a number of times with the above salt solution. The extract was protected from bright light to reduce photodecomposition of the pigments.

Preparation of Thin Layer Plates.

A slurry of the adsorbent was prepared by mixing the following: cellulose powder, 8 grams (MN cellulose powder 300, Brinkmann Instruments, Inc.); sugar, 2 grams (C & H sugar,

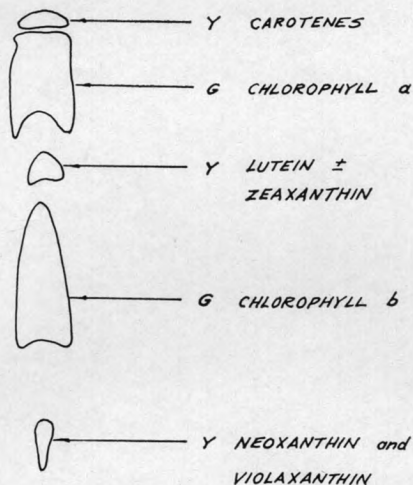


Figure 1. Chromatogram of plastid pigments

confectioners, powdered); 3% starch (potato, Baker Analyzed); and 50 ml. distilled water. The mixture was blended in a Waring Blendor for a few minutes, and well mixed to prevent lumpiness before application to the plate. The 200- × 200-mm. glass plates were washed with petroleum ether and air dried. The adsorbent was spread on the glass plates with a Brinkmann Instrument thin layer apparatus Model 250015. After applying the coating, the plates were dried at room temperature for 15 minutes and finally dried in an oven at 100° C. for 15 minutes.

Separation Procedure. One-tenth milliliter of the petroleum ether extract solution was spotted on the adsorbent coated plate. The plate was dried under a stream of nitrogen gas and placed in a tightly covered, wide-mouth, rectangular (230 × 230 × 50 mm.) jar containing approximately 300 ml. of developing solvent—0.5% *n*-propanol in petroleum ether. Nitrogen gas was passed through the jar to displace the air in the jar. The chromatogram was developed at 5° C. in the dark.

The development was stopped when the solvent front was about 18 cm. from the bottom of the plate.

RESULTS

Figure 1 shows the separation of the pigments in the following sequence from top to bottom, carotenes, chlorophyll *a*, lutein ± zeaxanthin, chlorophyll *b*, violaxanthin, and neoxanthin. Time to achieve separation was about 45 to 50 minutes.

For the identification of each pigment the colored zone was scraped off the plate, washed in acetone, and filtered through a cotton plug. The absorption spectrum for each colored zone was obtained on a recording spectrophotometer. The absorption spectrum obtained for each spot matched the spectra reported in the literature.

ACKNOWLEDGMENT

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