PARTIAL SEPARATION OF THE PLASTID PIGMENTS BY PAPER CHROMATOGRAPHY[1]

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A method for separation of small quantities of plastid pigments was desired, and paper chromatography seemed to be the best prospect among the available techniques. A search of the literature revealed only one direct mention of the application of paper chromatography to the separation of the plastid pigments. STRAIN (**3**), in a review article, stated that chlorophylls and xanthophylls can be separated by paper chromatography using aqueous ethanol as the developing solvent. In a private communication Dr. S. Aronoff has informed us that he obtained a separation of the plastid pigments by repeated development of the paper chromatogram in Skelly-B (petroleum ether). When tested in this laboratory neither of these two methods gave a complete separation of all visibly present fractions.[2]

In these investigations plastid pigments were extracted from soybean and other leaves with acetone in a Waring Blendor and transferred to Skelly-B in a separatory funnel. The Skelly-B solution was washed thoroughly with water to remove acetone and acetone-soluble impurities, then concentrated under vacuum. One tenth of a milliliter of the Skelly-B solution was placed at the origin (fig. 1) on a square of Whatman no. 1 filter paper (23 cm. × 23 cm.) which previously had been washed with Skelly-B and air dried. The filter paper was stapled together to form a cylinder which was placed in a tightly covered widemouth gallon jar containing ca. 100 ml. of developing solvent. The paper was air dried after development in each of the successive solvents.

Among the solvents and solvent combinations tested the following sequence gave the best results:

FIRST DIMENSION.—(a) Acetone: All the pigments moved with the solvent front and the development was stopped when the solvent reached the top of the original drop. This procedure served to collect the pigments from the original circular spot in a line front from which they could be more easily separated in the later steps. (b) Skelly-B: The carotenes followed

[1] Journal paper no. J-2128 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project no. 1139.

[2] A paper by Bauer (BAUER, L. Trennung der Karotinoide und Chlorophylle mit Hilfe der Papierchromatographie. Naturwiss. 39(4): 88. 1952.) became available after this paper was submitted for publication. Bauer separated the plastid pigments into nine fractions by two-dimensional paper chromatography, using petroleum solvents which are not readily available in this country.

the solvent front while the other visible pigments moved only slightly, if at all, from the origin. The development was stopped when the solvent was ca. 20 cm. from the bottom of the paper. This step brought the carotenes so far ahead of the other pigments that they did not interfere with further separations. (c) One volume per cent. n-propanol in Skelly-B: All pigments moved, but this treatment separated the remaining pigments into the following groups (top to bottom): Chlorophyll *a* overlapping the xanthophylls, chlorophyll *b*, and an unknown yellow pigment which appeared below and well separated from chlorophyll *b* in the chromatogram. The development

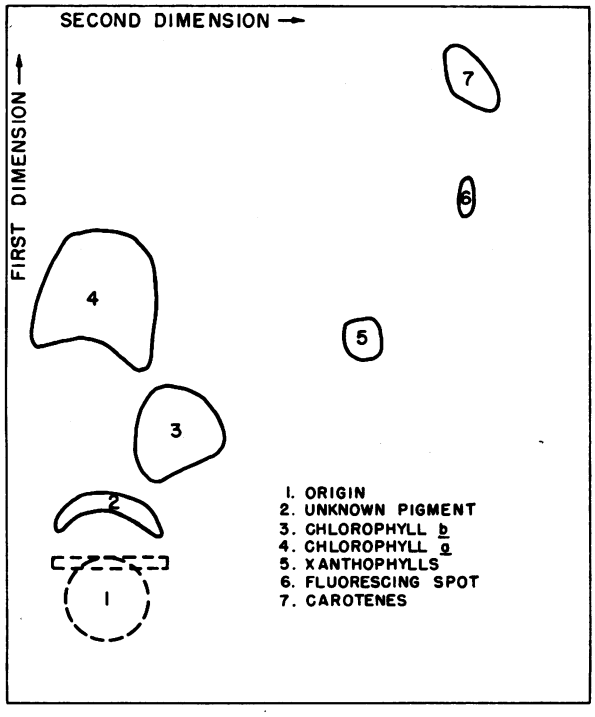

FIG. 1. Tracing of a paper chromatogram of soybean leaf pigments developed as described in the text.

was stopped when the solvent reached ca. 20 cm. from the bottom of the paper.

SECOND DIMENSION.—(d) Twenty-five volume per cent. chloroform in Skelly-B (the chloroform was thoroughly washed with water and dried with $CaCl_2$): The carotenes moved with the solvent front, the xanthophylls moved out of the chlorophyll *a*, which was almost stationary. Chlorophyll *b* moved, although much slower than the xanthophylls, and the unknown pigment did not move visibly. The development was stopped when the solvent had moved up ca. 16 cm.

After the completion of this development the chromatogram showed a distinct separation of the plastid pigments into six fractions: The carotenes, the xanthophylls, chlorophyll *a*, chlorophyll *b*, an unknown pigment, and a colorless but fluorescing compound found between the locations of the carotenes and the xanthophylls (fig. 1).

The identification of the unknown pigment was attempted by determining its absorption spectrum, which is shown in figure 2 together with the absorption spectrum of chlorophyll *b*. Absorption spectra of the carotene fractions and of chlorophyll *a* were also determined. The pigments were purified by the chromatographic technique described, redissolved in methanol and the absorption spectra measured with a Beckman spectrophotometer (Model DU).

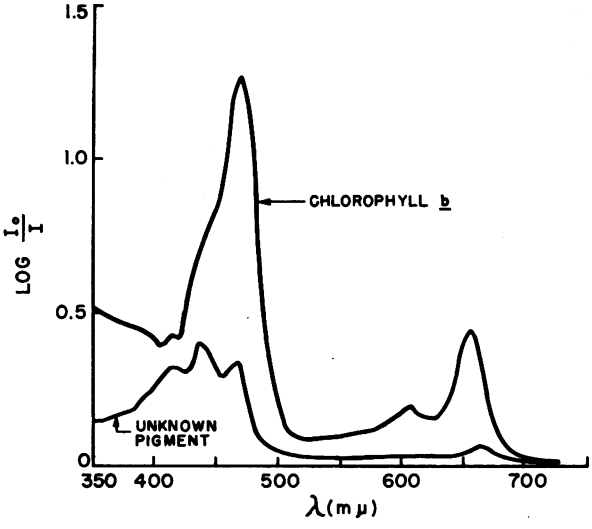

FIG. 2. Absorption spectra of chlorophyll *b* and the unknown pigment (neoxanthin?) dissolved in methanol.

The chlorophyll spectra conformed very closely to those reported by STRAIN and MANNING (**2**), and the absorption spectrum of the carotene fraction indicated that β-carotene was the main component. The unknown pigment had pronounced absorption maxima at 415, 437, and 467 mμ and a secondary maximum (fig. 2) at 662 mμ. The first three maxima indicate a carotenoid while the maximum at 662 mμ probably is due to impurities, possibly decomposition products of the chlorophylls. The unknown pigment may be neoxanthin, which was reported by STRAIN (**1**) to have absorption maxima at 434 and 464 mμ when dissolved in methanol and at 415, 437, and 467 mμ when dissolved in ethanol.

The method as outlined is a rapid qualitative method and it has definite possibilities of being made quantitative. Also, if some attention is given to

the details, the procedure makes a striking class-room demonstration. If one paper is started through the first dimension while a second, previously prepared, is started through the second dimension, a demonstration of the first and final steps can be completed in about one hour.

LITERATURE CITED

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3. STRAIN, H. H. Chromatographic separations. Anal. Chem. **21**: 75. 1949.