

September 1969

Brief 69-10425



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## Comparative Chromatography of Chloroplast Pigment

Methods for isolation of low-concentration pigments of the cocklebur species are described (1). The methods entail two-step chromatography so that the different sorption properties of the various pigments, in varying column parameters, can be utilized.

There are few systematic and reliable chromatographic procedures for complete resolution of the leaf pigments. Usually the chromatographic separations must be carried out empirically with various chromatographic systems until no further resolution is possible.

In order to provide more-comparable results from chromatography of the leaf pigments, separations with various adsorbents and wash liquids were studied. Columnar methods and thin-layer methods were employed under comparable conditions, and the separations were followed in relation to the loading of the systems with the pigment mixtures. The purposes included direct comparison of thin-layer and columnar techniques, determination of whether or not the major pigments may be resolved further, and confirmation of identities of the minor pigments. For the present, the more-promising chromatographic systems were utilized for separation of the pigments of a single species (*Xanthium*, or cocklebur) grown under reproducible greenhouse conditions.

Results indicate that many conditions influence separability of the chloroplast pigments by chromatography. These conditions include the nature and treatment of the sorbent, the solvent or wash liquid, the form in which the sorbent is employed, and the loading of the sorbent with the mixture. In the form of columns or thin layers, a given adsorbent provides reproducible capacity and selectivity for resolution of chloroplast pigments only when the same wash liquid is employed.

Various adsorbents exhibit different capacities and selectivities for the pigments, and they often provide different sequences of the separated pigments. Some adsorbents such as magnesia show variation in capacity and selectivity when made into thin layers from aqueous slurries. Very finely divided adsorbents, such as activated magnesia, may be employed both in columns and in thin layers if mixed with a nonsorptive filter aid.

With all the chromatographic systems tested, the lower the initial loading with the mixture, the greater the resolution. Conversely, the higher the loading, the poorer the resolution. With either underloading or overloading, minor constituents may not be detectable.

Readsorption of large fractions of the pigments, obtained from one chromatographic system, improves separation and detection of the minor leaf pigments. Readsorption with a second chromatographic system permits the separation of pigments such as lutein and zeaxanthin that were inseparable in the first system. For these chromatographic and rechromatographic procedures, columns are much more adaptable than thin layers and sheets of the adsorbent.

For preparation of quantities of the leaf pigments, columnar methods are more convenient than thin-layer and paper techniques. Separations are faster and more adaptable in columns than in thin layers or in paper.

The major pigments of cocklebur proved to be chlorophylls *a* and *b*, neoxanthin, violaxanthin, lutein, and  $\beta$ -carotene. The minor pigments are cryptoxanthin, zeaxanthin, and traces of  $\alpha$ -carotene, lutein epoxide, and (possible minute traces) zeaxanthin monoepoxide. There was no evidence that any

(continued overleaf)

of these pigments could be further resolved chromatographically.

**Reference:**

1. H. H. Strain, J. Sherma, and M. Grandolfo, *Comparative Chromatography of the Chloroplast Pigment* (Argonne National Laboratory, July 1967).

**Notes:**

1. These methods may interest researchers in plant pigments, photosynthesis, or energy-transfer.
2. Inquiries may be directed to:

Office of Industrial Cooperation  
Argonne National Laboratory  
9700 South Cass Avenue  
Argonne, Illinois 60439  
Reference: B69-10425

Source: H. H. Strain, J. Sherma,  
and M. Grandolfo  
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**Patent status:**

Inquiries concerning rights for commercial use of this innovation may be made to:

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