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Note

Separation of chloroplast leaf pigments by chromatography on starch and cellulose thin layers

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A very detailed study of the isolation and separation of the chloroplast pigments of leaves by paper and thin-layer chromatography (TLC) on various supports has been made by Sherma and co-workers[1–7]. Paper[1,3,5], cellulose (layers and sheets)[3–6], sugar[5], starch[5], siliceous adsorbents[2,5–7], alumina[5,6], magnesia[5], lime[5], polyamide[6] and some other supports[5] and various solvent systems were examined by one-dimensional, two-dimensional and radial chromatography. Mild adsorbents, such as cellulose, sugar and starch, are useful for the separation and recovery of chloroplast pigments as they will not alter chlorophylls and carotenoids (contrary to siliceous adsorbents, alumina, lime and magnesia[2,5,8]), but no complete separation of the principal pigments (chlorophyll *a* and *b*, violaxanthin, neoxanthin, lutein and *β*-carotene) has been obtained with the solvent mixtures employed[1,3–6]. Schneider[9] achieved a complete separation of the principal pigments on cellulose layers with methanol–dichloromethane–water (100:18:20) in the opposite sequence to that obtained with less polar solvent systems[3–6,8].

This paper presents our results on separations of leaf pigments on starch, cellulose and microcrystalline cellulose layers. Starch, a support which can be considered to be practically without adsorption properties, compared with cellulose, showed generally greater selectivity, especially for minor components of pigments.

EXPERIMENTAL

Thin layers were prepared from (i) commercial maize starch (Servo Mihalj, Zrenjanin, Yugoslavia), (ii) cellulose MN300 (Marcherey, Nagel & Co., Düren, G.F.R.) and (iii) microcrystalline cellulose (Merck, Darmstadt, G.F.R.).

(i) 40.5-g amount of maize starch and 4.5 g of gypsum were suspended in 60 ml of distilled water and the suspension was coated on 20 × 20 cm glass plates to a thickness of 0.4 mm with Desaga equipment. The thin layers were dried in air at room temperature.

(ii) A 15-g amount of cellulose MN300 was suspended in 100 ml of distilled water in a Waring blender and the suspension was applied to glass plates as described in (i). The air-dried layers were heated for 30 min at 110° before use.

(iii) A 15-g amount of microcrystalline cellulose was suspended in 65 ml of distilled water and the suspension was applied to glass plates as described in (i). The air-dried layers were heated for 30 min at 110° before use.

Spinach leaves, obtained from a greenhouse, were utilized for the isolation of pigments. Leaf extracts were prepared by a slightly shortened procedure of Strain *et al.*[1]. Freshly collected leaves (10 g) and 1.5 g of magnesium carbonate were placed in a mixture of 30 ml of acetone and 10 ml of light petroleum (b.p. 40–70°) and disintegrated with an Ultra-Turrax homogenizer. The suspension was centrifuged and the clear green supernatant was dried with anhydrous sodium sulphate and used for chromatography.

For saponification of green pigments 10 g of fresh leaves were homogenised with an Ultra-Turrax homogenizer with 40 ml of 20% potassium hydroxide solution. After 30 min, a mixture of 30 ml of acetone and 10 ml of light petroleum was added, and the suspension was homogenized again and transfered into a 250-ml separating funnel. The green suspension was diluted with 10 ml of light petroleum and 100 ml of 4% sodium chloride solution. The upper layer was separated, washed twice with 100-ml portions of distilled water, dried with anhydrous sodium sulphate and used for chromatography.

The isolation operations were carried out in the dark when ever possible.

The pigment extract was applied across the chromatographic plate as a strip with an automatic Desaga applicator [TNO (Delft, The Netherlands) system]. The chromatograms were developed in a rectangular glass TLC chamber, which contained about 50 ml of *n*-heptane–ethyl acetate–*n*-propanol (50:5:0.5) solvent mixture. The chromatograms were run in the dark at room temperature without previous saturation of the chamber with solvent vapour. The development times were 45–50 min for cellulose MN300 and starch and about 60 min for microcrystalline cellulose layers.

The separated zones were detected by visual observation and by colour reaction with hydrogen chloride vapour. The pigments eluted from separated zones were identified by their spectral absorption properties, determined with a Pye Unicam SP800B recording spectrophotometer. Absorption spectra of pigments eluted from minor zones were recorded in a 10-cm path-length cell, because of their low concentration.

RESULTS AND DISCUSSION

Complete separations of the principal pigments from primary or saponified extracts on both starch and cellulose layers with *n*-heptane–ethyl acetate–*n*-propanol (50:5:0.5) as solvent system were obtained (Figs. 1–3). Similar, but not as satisfactory separations were achieved when *n*-heptane was replaced with *n*-hexane or light petroleum (b.p. 70–110°). From the green extract, on starch layers were developed, in addition to the principal pigments, four yellow (y_1–y_4) and one green (g) minor zones (Fig. 1A); from the saponified extract, the principal pigments and five minor zones (y_1–y_5) were resolved (Fig. 1B). All principal pigments and a very poorly visible pheophytin fraction were separated from the green extract on microcrystalline cellulose

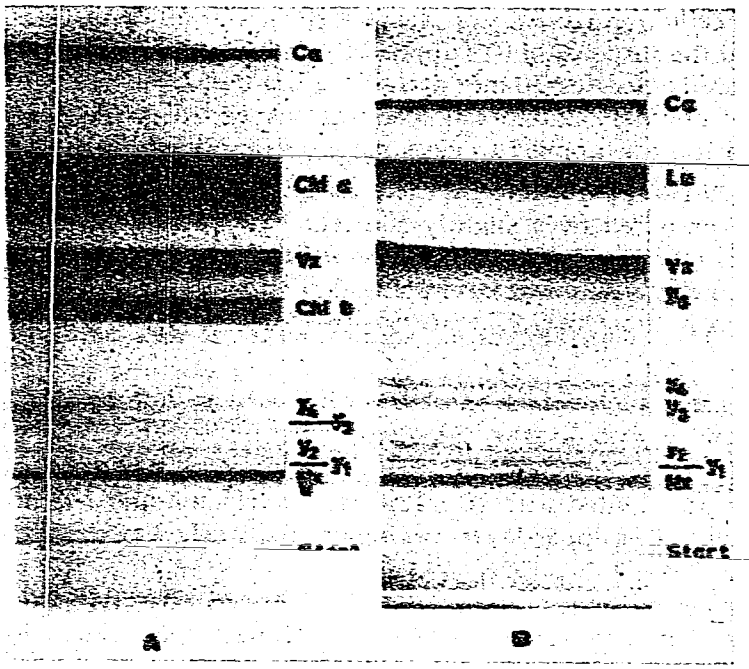

Fig. 1. Separation of leaf pigments from (A) primary and (B) saponified extracts om maize starch thin layers. Chl a = chlorophyll *a*; Chl b = chlorophyll *b*; Lu = lutein; Vx = violaxanthin; Nx = neoxanthin; Cx = β-carotene; y = yellow minor pigment; g = green minor pigment.

layers (Fig. 2A); from the saponified extract, the principal pigments and two minor zones (y_1 and y_2) were resolved (Fig. 2B). Under the same conditions, on cellulose MN300 layers only the principal pigments were developed, from the both green and saponified extracts (Fig. 3).

The separations presented in Figs. 1–3 were carried out in parallel with the same extract and the same amounts of pigments solution, so that the appearance of the different minor zones on starch and cellulose layers is due to the different support properties. Hence starch is a more selective support than cellulose.

The spectral absorption properties of the resolved yellow minor pigment fractions are presented in Table I. The principal pigments have their characteristic spectra and are not listed in Table I. The yellow minor zones resolved on starch layers, designated y_1 and y_2, turned blue-green, y_3 and y_4 turned blue and y_5 remained yellow under the influence of hydrogen chloride. Zone y_1 resolved on microcrystalline cellulose layers turned blue and y_2 remained yellow under the infiuence of hydrogen chloride.

The hypsochromic shift with hydrogen chloride of about 20 nm showed that y_1 and y_2 fractions separated on starch layers are monoepoxides. The y_1 fraction is less stable than the y_2 fraction. Its conversion to the furanoxide form occurs very rapidly. The y_3 fraction is a mixture of a diepoxide and a difuranoxide, and the y_4

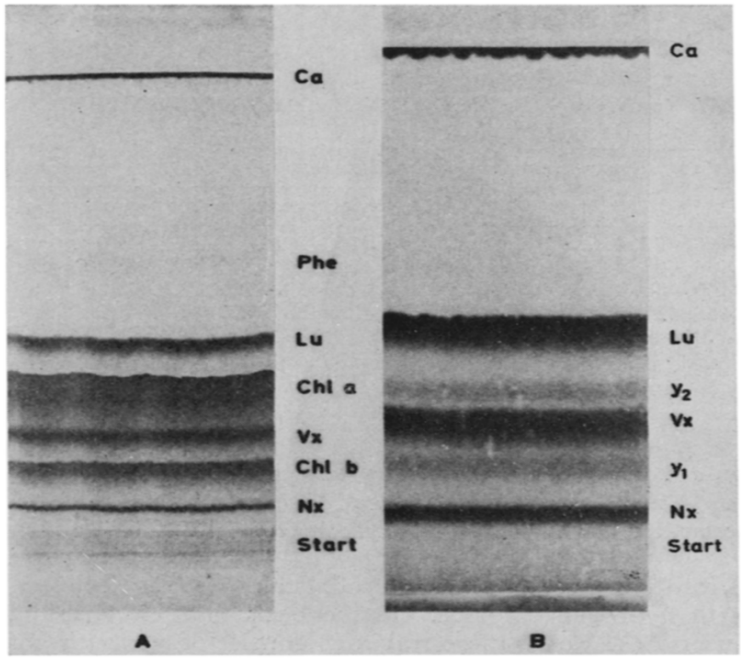

Fig. 2. Separation of leaf pigments on microcrystalline cellulose thin layers. Symbols and abbreviations as in Fig. 1.

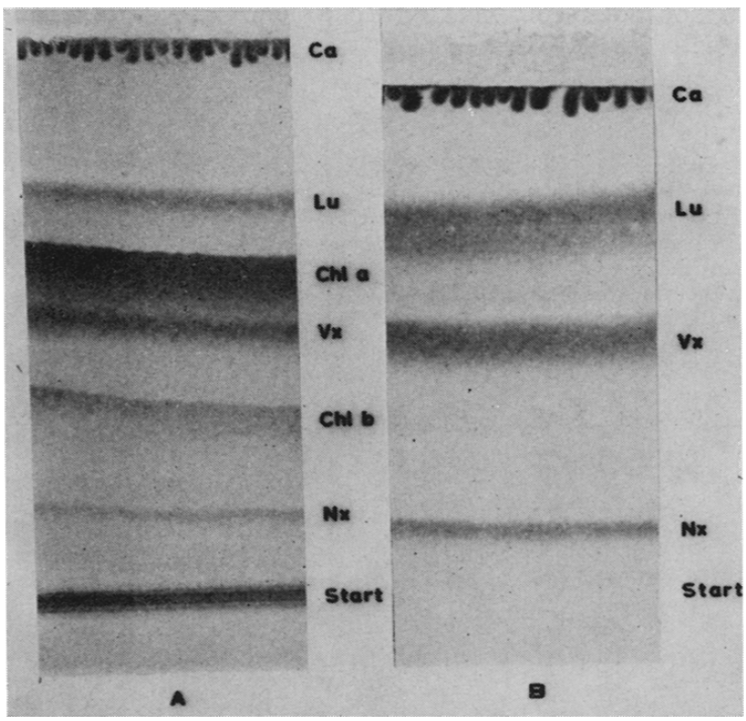

Fig. 3. Separation of leaf pigments on cellulose MN300 thin layers. Symbols and abbreviations as in Fig. 1.

TABLE I

VISIBLE ABSORPTION MAXIMA (nm) OF MINOR CAROTENOIDS DETERMINED IN ABSOLUTE ETHANOL

Layer	*Zone*	*Absorption maxima (nm)*	*Epoxide test*	
			Absorption maxima (nm)	*Colour*
Starch	y_1	468, 439, 416	446, 420, 400	Blue-green
	y_2	466, 438, 415	448, 421, 399	Blue-green
	y_3	469, 443, 423, 400, 378	424, 400, 378	Blue
	y_4	465, 437, 415	425, 400, 377	Blue
	y_5	470, 440, 425, 402, 380	No effect	Yellow
Microcrystalline cellulose	y_1	469, 440, 421, 400, 380	448, 425, 400, 379	Blue
	y_2	470, 440, 424, 401, 380	No effect	Yellow

fraction is a diepoxide. The y_1 zone resolved on microcrystalline cellulose layers had a very similar spectrum to the y_3 zone separated on starch layers and is probably a mixture of all minor zones separated on starch layers, which reacted with hydrogen chloride.

The y_5 and y_2 fractions resolved on starch and microcrystalline cellulose layers, respectively, did not react with hydrogen chloride. On the basis of their virtually identical visible absorption spectra (Table I), these fractions consist of a mixture of two carotenoids, which possessed chromophores of 9 and 7 conjugated double bonds[10]. Their positions on the chromatograms and the fact that they could not be eluted from the support with non-polar solvents (light petroleum or *n*-hexane) indicates their polarity (elution with ethanol is complete).

REFERENCES

1 H. H. Strain, J. Sherma, F. L. Benton and J. J. Katz, *Biochim. Biophys. Acta*, 109 (1965) 1, 16 and 23.
2 H. H. Strain, J. Sherma and M. Grandolfo, *Anal. Chem.*, 39 (1967) 926.
3 J. Sherma and G. Zweig, *J. Chromatogr.*, 31 (1967) 439.
4 J. Sherma and G. Zweig, *J. Chromatogr.*, 31 (1967) 589.
5 H. H. Strain, J. Sherma and M. Grandolfo, *Anal. Biochem.*, 24 (1968) 54.
6 J. Sherma and G. S. Lippstone, *J. Chromatogr.*, 41 (1969) 220.
7 J. Sherma, *J. Chromatogr.*, 52 (1970) 177.
8 Z. Šestak, *Photosynthetica*, 1 (1967) 269.
9 H. A. W. Schneider, *J. Chromatogr.*, 21 (1966) 448.
10 B. H. Davies, in T. W. Goodwin (Editor), *Chemistry and Biochemistry of Plant Pigments*, Vol. 2, Academic Press, London, 2nd ed., 1976, p. 125.