CHROM. 4612

The chromatographic separation of magnesium protoporphyrin IX dimethyl esters from zinc protoporphyrin IX dimethyl esters*

In our present studies that are aimed at demonstrating the enzymatic insertion of magnesium into porphyrins, we incubate various porphyrins and magnesium salts with a chloroplast suspension (*e.g.*, ref. 1), extract and esterify the metalloporphyrins, and then attempt to isolate magnesium protoporphyrin IX dimethyl ester from the pigment mixture. Plant homogenates have been shown to have a zinc chelatase enzyme[2], and each time we have performed the experiment described above using protoporphyrin IX as a substrate, we obtain a sizable quantity of zinc protoporphyrin IX dimethyl ester. We subsequently observed that synthetic magnesium protoporphyrin IX dimethyl ester and zinc protoporphyrin IX dimethyl ester were not resolvable using conventional sucrose column chromatography with isopropanol–light petroleum[3] or benzene–light petroleum[4] as developer. Therefore, if some Mg-protoporphyrin IX dimethyl ester were present in the (esterified) pigments extracted from the incubation mixture, it would not have separated from the large quantity of Zn-protoporphyrin IX dimethyl ester.

The chromatographic procedure described below was developed and found to be very suitable for separating Mg- and Zn-protoporphyrin IX dimethyl ester (as well as the free base) in 1 mg quantities, even when one part (or less) of Mg-protoporphyrin IX dimethyl ester was mixed with 99 parts (mole basis) of Zn-protoporphyrin IX dimethyl ester.

The metalloporphyrin esters (1 mg or less) were dissolved in a minimal volume (*e.g.*, 1 or 2 ml) of benzene, diluted (just prior to the addition to the sucrose column) with 3 volumes of light petroleum (b.p. range 35–60°), and then added to a 20 × 250 mm sucrose (Domino 10X brand containing 3 % (w/w) cornstarch) column which was packed dry, under vacuum and run under vacuum. Just prior to the column's running dry a solution of benzene–light petroleum (1:3, v/v) was added to completely wash the metalloporphyrin esters onto the column (about 1/2 to 1 h of washing with the 1:3 (v/v) benzene–light petroleum solvent is sufficient). Just before the solvent head was depleted, a developer of 0.5 % (v/v) pyridine in light petroleum was added. The zinc protoporphyrin IX dimethyl ester rapidly moved away from the magnesium porphyrin. Fig. 1 shows the typical separations achieved with this chromatographic system of a mixture of Mg and Zn-protoporphyrin IX dimethyl esters and the free base dimethyl ester.

If the Mg- and/or Zn-protoporphyrin IX dimethyl esters are further mixed with plastid pigments, the latter can be readily removed from the metalloprotoporphyrin IX dimethyl esters during the development with the benzene–light petroleum solvent. (This result is shown in Fig. 2.) The metalloporphyrin band (*i.e.*, a mixture of Mg- and Zn-protoporphyrin IX dimethyl esters) is then removed and rerun on another sucrose column (as above with the two solvent systems) because the pyridine–light petroleum solvent system moves the plastid pigments down the column, contaminating the Mg-protoporphyrin IX dimethyl ester band.

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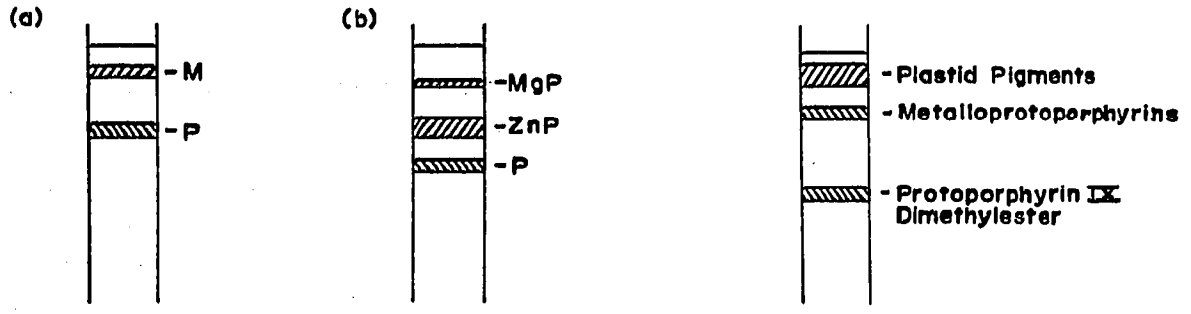

Fig. 1. Separation of a mixture of the magnesium and zinc chelates and the free base of protoporphyrin IX dimethyl ester by sucrose column chromatography. (a) Column developed approximately 1/2 h with a solution of benzene–light petroleum (1:3, v/v); *M* = metalloporphyrin esters, P = protoporphyrin IX dimethyl ester. (b) Distribution of pigments when column is treated as in (a) above, then developed with 0.5% (v/v) pyridine in light petroleum. MgP = magnesium protoporphyrin IX dimethyl ester, ZnP = zinc protoporphyrin IX dimethyl ester, P = protoporphyrin IX dimethyl ester.

Fig. 2. Separation of metalloprotoporphyrin IX dimethyl esters from leaf pigments and protoporphyrin IX dimethyl ester on a sucrose column using benzene–light petroleum (1:3, v/v) as developer.

After the desired separation of Mg- and Zn-protoporphyrin IX dimethyl esters was achieved, the chromatographic bands were manually extruded, and the pigments were washed into diethyl ether. The ethereal pigment solutions were evaporated to dryness and the residue was dissolved in fresh diethyl ether for spectrophotometry. The visible absorption spectra of our synthetically prepared Mg- and Zn-protoporphyrin IX dimethyl esters in ether are shown in Fig. 3. (The metalloporphyrin absorption spectra were extremely reproducible and unique to the two metalloporphyrins in anhydrous diethyl ether.) The absorption spectra of the metalloporphyrins which comprised the chromatographic bands indicated in Fig. 1 were identical with those of the synthetic starting materials when the pigments were recovered from the chromatographic column in the manner described above.

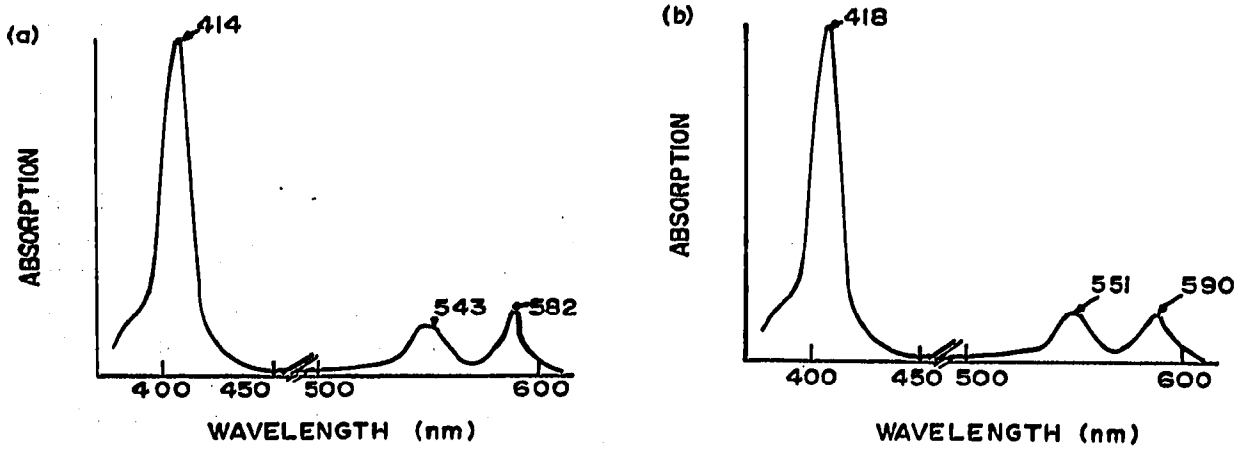

Fig. 3. Visible region absorption spectra of synthetic (a) zinc protoporphyrin IX dimethyl ester and (b) magnesium protoporphyrin IX dimethyl ester in anhydrous diethyl ether as recorded on a Cary Model 14 spectrophotometer.

Protoporphyrin IX dimethyl ester was obtained commercially (Sigma Chemical Company, St. Louis, Mo., U.S.A.). Magnesium was quantitatively inserted into protoporphoryin IX dimethyl ester by the method of BAUM *et al.*[5]. Zinc was quantitatively incorporated into protoporphyrin IX dimethyl ester by dissolving the porphyrin in methanol and adding solid zinc nitrate. After waiting approximately 5 min, the methanol solution was added to an equal volume of diethyl ether, and then the methanol and residual zinc nitrate were washed out with distilled water.

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Department of Chemistry, State University of New York, College of Arts and Science, Plattsburgh, New York 12901 (U.S.A.)

S. J. BAUM
R. K. ELLSWORTH

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A two-dimensional pipette for sample application in preparative thin-layer chromatography

The undisputed necessity of thin, straight, and homogeneous sample application bands in preparative thin-layer chromatography prompted us to construct a two-dimensional pipette based on the principle employed in the Desaga broad band pipette[1]. Two identical stainless steel plates were machined and hand-lapped with abrasive so that surface S was as plane as possible and knife-edge K was razor-sharp, nick-free, linear, and unwarped (Fig. 1). (Poor results from a glass prototype, owing

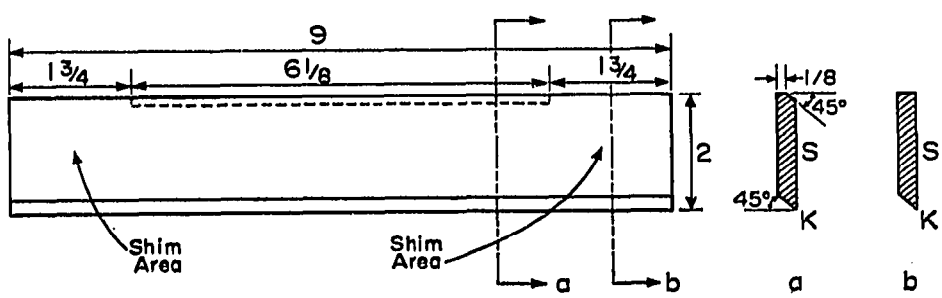

Fig. 1. Two-dimensional pipette. Dimensions in inches.