Simple Method for Separation of Milligram Quantities of Protochlorophyll *a* from Seed Oils in Extracts of Whole Pumpkin Seeds[1]

During our study of the enzyme protochlorophyllase (1), as substrate for the reaction we used protochlorophyll *a* derived from pumpkin seeds ("Big Tom type," W. Atlee Burpee Co., Philadelphia, Pa.) because they are a rich source of the compound. To avoid the manual peeling of lots of 100–1000 inner seed coats in order to obtain the protochlorophyll relatively free of seed oils, we used the following procedure:

150 gm seeds was ground in a Waring Blendor with 200 ml cold (0°) reagent-grade acetone at low speed for 1 min, then at high speed for 2 min. The resulting homogenate was filtered under vacuum through 1 layer of Whatman No. 1 filter paper, the filtrate was added to 200 ml light petroleum (35–60° fraction), and the acetone was washed free by repeated distilled water rinsings of the light petroleum layer. The resulting light petroleum solution was dried briefly with solid, anhydrous Na_2SO_4, decanted, and then concentrated under vacuum at 30° until a very oily consistency was obtained. To this oily residue an equal volume (usually 30–50 ml) of light petroleum was added.

To facilitate matters, during workup of the pigment solution, a 2.5 cm $\times$ 10 to 15 cm column of silica gel (silica gel G for thin-layer chromatography, E. Merck A. G., Darmstadt; Brinkman Instruments, Inc., Westbury, New York) was tightly packed under highest achievable vacuum from a water aspirator. To this column, which was being run under maintained high vacuum, was added the light petroleum solution containing the pigmens and oils (see above). Just prior to running dry, 20 ml light petroleum was added to the column. Again before the column was allowed to dry, a solution of 30% (v/v) diethyl ether in light petroleum was added. This solution washed the seed oils and carotenes free from the protochlorophylls, which remained strongly adsorbed at the top of the chromatographic column.

At the end of a two to three hour washing period the dark-green protochlorophyll band on top of the column began to move down the column. This observation signaled the end of the washing period. The top dark-

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green layer containing protochlorophyll was manually extruded from the column, and then added to 50 ml diethyl ether. After brief stirring, this suspension was filtered, under vacuum, through Whatman No. 1. The filtrate was then evaporated to dryness at 30° under vacuum. No noticeable seed oils were present in the residue (although some carotenoids were observed in the visible absorption spectrum).

For further purification (including removal of carotenoid pigments) of the protochlorophyll, depending on the pigments desired, one may subject the final residue obtained to conventional sucrose column chromatography using the developer of Jones (2) or of Strain *et al.* (3).

Using the procedure described in this report and then chromatographing the pigments obtained therefrom on sucrose columns following the method of Strain *et al.* (3), we obtained approximately 4 mg protochlorophyll *a* (a mixture of mono- and divinyl compounds as described by Jones (2)) and 2.5 mg protopheophytin *a* (also a mixture of mono- and divinyl compounds). The 4-vinylprotochlorophyll *a* and protochlorophyll *a* were further separated by a second pass on a sucrose column as described by Jones (2). The absorption maxima and ratios of peak height intensities obtained for these two protochlorophyll *a* pigments in anhydrous, peroxide-free diethyl ether, which are presented in Table 1, agreed well with

TABLE 1
Spectroscopic Properties of Protochlorophyll *a* and 4-Vinylprotochlorophyll *a* in Ether[a]

Pigment	Absorption (nm)			
Protochlorophyll *a*	623 (12.1)	570 (4.5)	534 (2.1)	432 (100)
4-Vinylprotochlorophyll *a*	624 (11.1)	574 (6.2)	536 (2.6)	438 (100)

[a] Relative absorbances of the bands are given in parentheses; Soret band taken as 100.

published values (2). These two pigments ran identically with authentic protochlorophyll *a* and 4-vinylprotochlorophyll *a* (which were extracted from peeled pumpkin seeds and then purified through the series of two sucrose column chromatographic separations mentioned above) on sucrose and (4) cellulose thin layers (5). The visible and thin-layer chromatographic data obtained suggested that the protochlorophylls prepared by the procedure described in this report were authentic.

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